

**Investigation into the cause(s) of a mass mortality of a long-lived  
species in a Provincial Park and an evaluation of recovery strategies.**

**by**

Donnell Marie-Leah Gasbarrini

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science (MSc) in Biology

The Faculty of Graduate Studies  
Laurentian University  
Sudbury, Ontario, Canada

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**THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE**  
**Laurentian University/Université Laurentienne**  
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Investigation into the cause(s) of a mass mortality of a long-lived species in a Provincial Park and an evaluation of recovery strategies	
Name of Candidate Nom du candidat	Gasbarrini, Donnell	
Degree Diplôme	Master of Science	
Department/Program Département/Programme	Biology	Date of Defence Date de la soutenance September 23, 2016

**APPROVED/APPROUVÉ**

Thesis Examiners/Examineurs de thèse:

Dr. David Lesbarrères  
(Co-Supervisor/Co-directeur de thèse)

Dr. Jackie Litzgus  
(Co-Supervisor/Co-directrice de thèse)

Dr. Edward Morris  
(Committee member/Membre du comité)

Dr. Patricia Chow-Fraser  
(External Examiner/Examineur externe)

Approved for the Faculty of Graduate Studies  
Approuvé pour la Faculté des études supérieures  
Dr. David Lesbarrères  
Monsieur David Lesbarrères  
Dean, Faculty of Graduate Studies  
Doyen, Faculté des études supérieures

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## **Abstract**

Mass mortality events (MMEs) are rapidly occurring and localized events, and have been reported to remove up to 90% of individuals in a population. MMEs can be especially damaging to population persistence for long-lived species, such as chelonians. While MMEs have been regarded as rare events, they are predicted to occur with increased frequency as environmental stochasticity associated with climate change increases. Unfortunately, a limited understanding of the causes and consequences of MMEs remains. In the current thesis, I investigated the potential causes of an acute MME of at-risk Blanding's turtles (*Emydoidea blandingii*) at Misery Bay Provincial Park on Manitoulin Island, Ontario in which approximately 50% of the population succumbed to mortality, and used population viability analyses (PVAs) to examine strategies to recover the population. Because the park includes relatively pristine habitat in which most of the regular anthropogenic threats to turtles are absent, the hypotheses I tested to explain the mortality considered natural threats, including disease, failed overwintering, and predation in the winter and active seasons. I determined that the most likely cause of death was a large-scale predation event, which received support from several lines of evidence, including the presence of predators within the park, a failed predation attempt on a live Blanding's turtle, and the meticulous destruction of a turtle decoy stationed where carcasses were found. The recovery strategies examined included nest protection, introduction of juveniles, introduction of adults, and a nest protection plus introduction of juvenile combination strategy. PVAs determined that the most effective recovery strategy for this population would be a combination of nest protection and the annual introduction of 25 two-year-old females for a period of 50 years. The information gained through my study has led to the recommendation of appropriate conservation strategies for this population, and will aid in the management of future MMEs elsewhere.

## **Acknowledgements**

From beginning to end, this project has been an incredible adventure that wouldn't have been nearly as interesting or successful without the many, many people that played so many different roles. I would like to start by thanking my exceptionally supportive co-supervisors, Dr.

Jacqueline Litzgus and Dr. David Lesbarrères. Your support made this project possible, and your guidance, advice, comments, and edits were always constructive and insightful, helping the Murder Mystery at Misery Bay to grow and form into this thesis. Thank you for never doubting in me or my abilities, and for your constant patience and enthusiasm throughout the past two years.

I would also like to thank my committee members, Ed Morris and Anna Sheppard. From spending time with me on my first few days on Manitoulin Island teaching me how to find, capture, and process turtles, to sharing gear and resources, your contributions are greatly appreciated. Thank you for taking time out of your busy schedules to meet with me to discuss the project, edit various proposal and thesis drafts, and for including me in learning opportunities. Ed, thank you for believing that I had everything I needed to complete this project “between my two ears.” Anna, thank you for spending so much time away from your family to help me in the field, every day in the field with you was memorable. Thank you for teaching me so much about turtles, birds, and odonates, and for teaching me even more about this crazy thing called life.

A special thanks to everyone who helped me in the field, especially Amber Kolodzik, Michael Hawton, and Sean Hudson, and also to Matt Keevil, Chris Blythe, Greg Rand, Will Kershaw, Alanna Smolarz, Jacqueline Litzgus, David and Eloïse Lesbarrères, Erin Calder, Joe Bienentreu,

Damien Mullin, and Ron Gould. Thank you to the Herp Lab members (past and present), Kelsey Marchand, Seany-B, Matt Keevil, Geoff Hughes, Joe Bientreau, Hannah McCurdy-Adams, Kiyoshi Sasaki, Michael Colley, Patrick Moldowan and Amanda Bennett for always being supportive and willing to talk about all things turtle, and for keeping the bar raised so high. Thank you to Lexi Sumner for introducing me to the best of Sudbury, and for all of the laughs that we shared throughout our time at 147 Adie Street.

A big thank you extends to Colleen Bobbie for her statistical support and for sharing her R expertise, and also to Samantha Grant and Trent University, for her aid in completing the disease analysis.

I was extremely fortunate to have met so many wonderful people on Manitoulin Island who had a special interest in this project. Thank you to Todd, Sean, Amanda, Jill, Justin, and Nick Secord, and to Gaynor and Floyd Orford, for welcoming me into their homes and families, and for always being kind, willing to help, and curious. I truly enjoyed my time at the cabin in the woods and on the farm. A thank you also extends to Jamie and Dorothy McFadden at Lake Wolsey Cabins. Thank you to the Friends of Misery Bay, particularly Gaynor Orford, for the support throughout the past two years.

I would like to thank all of my family and friends, especially my parents, Geraldine McDonnell and Gabe Gasbarrini, and my grandparents, Gerald McDonnell and Dan Gasbarrini, whose support and guidance have been invaluable throughout all of my schooling. I would also like to thank my parents, siblings, Dane, Anastasia, and Shea Gasbarrini, and friends, Stephanie

Hoekstra, Theresa Kim, Nicole Fyall, Jen Rowsell, and Kati Doma for visiting me on the Island.

It was a pleasure to share my experiences with each and every one of you.

I would like to extend a very special thank you to Jesse Jarvis for his willingness to edit my work, discuss ideas and challenges, and for his never-ending support and encouragement.

Finally, thank you to my role model, boss, mentor, and friend Peter Klose for inspiring me to try to save the world.

Thank you to all of the supporting agencies that helped to fund this project, Ontario Ministry of Natural Resources and Forestry Species at Risk Stewardship Fund, Ontario Parks, and Laurentian University.

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## **General Introduction**

### **Mass Mortality Events**

While superficially counterintuitive, death of individuals and extinction of species are essential to the continuous persistence and diversification of life. Historically, the Earth has experienced five mass extinction events, characterized by paleontologists as the loss of more than three-quarters of the Earth's species in a geologically short time interval, and we may currently be experiencing a sixth mass extinction (Dirzo et al., 2014; Barnosky et al., 2011; Wake and Vredenburg, 2008). The current extinction crisis is unique in that it is occurring at an unprecedented rate, and humans can be firmly held accountable (Dirzo et al., 2014; Thomas et al., 2004). Such crises have led to the emergence of conservation biology as a scientific field, aiming to support and preserve natural populations and species that are in decline and heading towards permanent peril, as humans feel an ever-growing responsibility to counter the damage being done to ecosystems (Soulé, 1985). While many populations are undergoing declines that can be described on the scale of centuries and decades, others have experienced declines in a much more dramatic fashion, with amphibians and birds showing highest susceptibility to human impact among vertebrates, whereas reptiles and mammals are considered intermediately threatened worldwide (Dirzo et al., 2014; Millennium Ecosystem Assessment, 2005). Additionally, species inhabiting freshwater and marine ecosystems are at particularly high risk of decline when compared to those in terrestrial systems (Böhm et al., 2013).

Mass mortality events (MMEs) are rapidly-occurring and localized events that result in the death of a large proportion of individuals in a population over a relatively short period of time (Fey et al., 2015). MMEs can simultaneously affect all life stages and can quickly result in up to 100%

mortality in a population (Lande, 1993; Reed et al., 2003; Jáurez et al., 2011). These events have likely occurred throughout human and pre-human history, but there remains little information about the causes and repercussions of MMEs in ecological systems (Wake and Vredenburg, 2008). Considering the pace and extent by which populations are affected by MMEs, and our limited understanding of these phenomena, it is important that MMEs are reported in the primary literature rather than only in small-scale news venues. Disseminating such information will enable a better understanding of the causes and consequences of MMEs, and allow the implementation of preventative and mitigation strategies (La and Cooke, 2011).

There is at least some documentation of MMEs in all classes of vertebrates (Fey et al. 2015). While the authors disclose that the caveats of small sample sizes must be considered when interpreting the results, the magnitude of MMEs with respect to number of deaths per event in reptile populations has increased since the 1970s (Fey et al., 2015). This increasing magnitude of MMEs in reptile populations also coincides with a documented global decrease in reptile populations (Böhm et al., 2013; Fey et al., 2015; Gibbons et al., 2000).

### **Turtles in Ontario**

Of all freshwater taxa, freshwater reptiles may be the most at-risk, as nearly half of all species are classified as threatened or near-threatened (Collen et al., 2014). Freshwater turtle declines are particularly evident in Ontario, as the Committee on the Status of Species at Risk in Ontario (COSSARO) classifies seven of the province's eight turtle species as at risk. These provincial statuses range from special concern, in the case of the snapping turtle (*Chelydra serpentina*), the eastern musk turtle (*Sternotherus odoratus*) and the map turtle (*Graptemys geographica*), to

threatened, as in the Blanding's turtle (*Emydoidea blandingii*), and endangered, in the case of the spiny softshell (*Apalone spinifera*), spotted turtle (*Clemmys guttata*) and the wood turtle (*Glyptemys insculpta*). The most prevalent threats to Ontario's turtle species result directly from human development and activities, and include habitat loss and fragmentation, population isolation, vehicular collisions resulting in road mortality, poaching, and subsidized predation (Browne and Hecnar, 2007; Beaudry et al., 2008; Miller and Blouin-Demers, 2011; Bennett and Litzgus, 2014; Gibbons et al., 2000).

Turtle species are generally known to have slow population recovery rates in response to increased mortalities, as they lack density-dependent responses necessary to recover, such as increased survival of young, earlier age of maturity, or greater reproductive output (Brooks et al., 1991). Alarming, Congdon et al. (1994) reported that an increase in annual adult mortality of just 10% in a population with no density-dependent compensation would result in a halving of the total number of adults in that population in fewer than 20 years. For these reasons, natural or anthropogenic loss of adult turtles can have overwhelming and long-term effects on population persistence.

### **The Blanding's Turtle**

Blanding's turtles are the only living representation of the genus *Emydoidea*. Populations of Blanding's turtles exist in eastern Canada and the northeastern USA (Congdon and van Loben Sels, 1993; COSEWIC, 2005). In Canada, they occur in discrete populations throughout southern and south-central Ontario and the extreme south of Quebec (the Great Lakes/St. Lawrence populations; Figure 0.1), as well as an isolated population in Nova Scotia (Standing et al., 1999;

COSEWIC 2005). As a long-lived and late-maturing reptilian species, Blanding's turtles have been known to endure lifespans in excess of 80 years, and typically require approximately 15 years to reach sexual maturity (Science Daily, 2016; COSEWIC, 2005; Congdon and van Loben Sels, 1993). However, populations of Blanding's turtles in the northern extent of their range experience delayed sexual maturity up to approximately age 25 years, increasing susceptibility to threats (Congdon et al., 2001; Bury and Germano, 2002; COSEWIC, 2005). While remarkable, these characteristics of their life history hinder their ability to recover from abnormally high adult mortality rates (Congdon et al., 1994; Brooks et al., 1991). Canadian populations have experienced significant declines, leading to the designation of the Great Lakes/St. Lawrence populations of Blanding's turtles as threatened by COSEWIC, and the status of the Nova Scotia population escalated from threatened to endangered upon the most recent reassessment in 2005 (COSEWIC, 2005). Each of the previously stated threats to Ontario's turtles are known to affect Blanding's turtles throughout their Ontario range, are intimately associated with human development and activities; however, a recent MME of Blanding's turtles has been identified in a seemingly pristine and protected habitat with little human influence.

### **Trouble in Paradise**

Misery Bay Provincial Park (MBPP; Figure 0.2) is a 1,100-ha Nature Reserve class of provincial park on the southern shore of Manitoulin Island, Ontario. The two closest communities are Evansville, 14.2 km to the east, and Silverwater, 14.7 km to the west. MBPP is on the opposite end of the island from both a swing bridge and a ferry, which allow access between mainland Ontario and the island. For these reasons, MBPP is a relatively quiet park, attracting just over 3,000 visitors annually (May-October; Orford, pers. comm., 2014.). The property is considered

provincially significant because of the expanses of flat limestone bedrock known as alvar, and the large wetland associated with the Lake Huron coast (OMNR, 1996). Existing within a substantial buffer of undeveloped private land and natural reserves between populated communities, and on the relatively pristine coast of Lake Huron, MBPP includes thriving and minimally disturbed habitat (Cvetkovic and Chow-Fraser, 2011; Sheppard, 2014a, b). The typical threats to turtles are minor or virtually absent in MBPP, and yet 63 turtles (53 Blanding's turtles and 10 painted turtles (*Chrysemys picta*)) were found dead without obvious cause. Most of the remains were only the plastron and carapace sections of the shell, lacking skulls or small bones associated with limbs. Extensive decomposition of the carcasses had occurred by the time they were found, indicating that some time had passed since the death of these animals, but the exact timeline of the mortality event was unclear.

### **Background Investigation**

This extensive mortality event generated interest and concern among Ontario Parks Ecologists, who focused on the Blanding's turtle population at MBPP during the 2013 field season. While Blanding's turtles were known to exist in MBPP prior to 2013, the population had never been monitored, and consequently population history and size estimates are non-existent. The Ontario Parks Ecologists conducted systematic and targeted surveys and radio telemetry studies in 2013 throughout the large wetland adjacent to Misery Bay proper as well as several small wetlands and vernal pools just west of Misery Bay, in an effort to find live and dead Blanding's turtles (Sheppard, 2014a). A total of 30 live Blanding's turtles were captured, marked (by shell notching; Cagle 1939), and measured during the 2013 field season (Sheppard, 2014a), and six were equipped with transmitters so that radio telemetry studies could be carried out to determine

home range sizes and areas of importance (Sheppard, 2014a, b). Upon completion of the 2013 field season, the project was adopted by Andrea Mendler, an undergraduate student in Forensic Science at Laurentian University, Sudbury, Ontario.

Mendler investigated the likelihood of predation and disease as causes of mortality through the 2013-2014 school year. The remains of dead turtles were examined for signs of predation, and an objective scoring system was developed to determine severity of markings on the skeletons. Ultimately, the results of the predation hypothesis were inconclusive (Mendler, 2014). The living population of Blanding's turtles at MBPP was examined for disease, namely viral copies of an FV3-like virus (indicative of ranavirus), through tissue samples, oral and cloacal swabs (N = 4) and toe clips of varying frog species (N = 15; Mendler, 2014). Desiccated skin and small bone samples were collected from carcasses and were also tested for ranavirus. No viral copies of an FV3-like virus were identified in chelonian samples, and were only detected in one Green Frog (*Lithobates clamitans*) toe-clip sample, but at levels below those indicative of an active case of the disease (Mendler, 2014). Mendler (2014) recommended that samples be taken from symptomatic turtles in future studies, but none have been encountered to date. Following Mendler's preliminary investigation, I undertook a more detailed two-year study.

## **Objectives**

One of the main objectives of my study was to determine the cause of the mortality of the Blanding's turtles in MBPP. I examined several potential factors, including disease, predation, and failed overwintering (including winter predation, acidosis, and freezing), to unveil aspects of the relationship between Blanding's turtles, their predators, and their habitat in MBPP. Whether this mortality event resulted from anthropogenic or natural influences, gaining insight into the factors involved will aid in identifying additional, and possibly previously unknown, threats to Blanding's turtles in Ontario. In addition to determining the cause of the MBPP MME, I used quantitative models to evaluate the effect of this MME on the MBPP Blanding's turtle population over time, and to make informative recommendations to managers regarding conservation strategies that could be employed. The results of my study will be informative for the management of future MMEs, and for the conservation and recovery of the study population.

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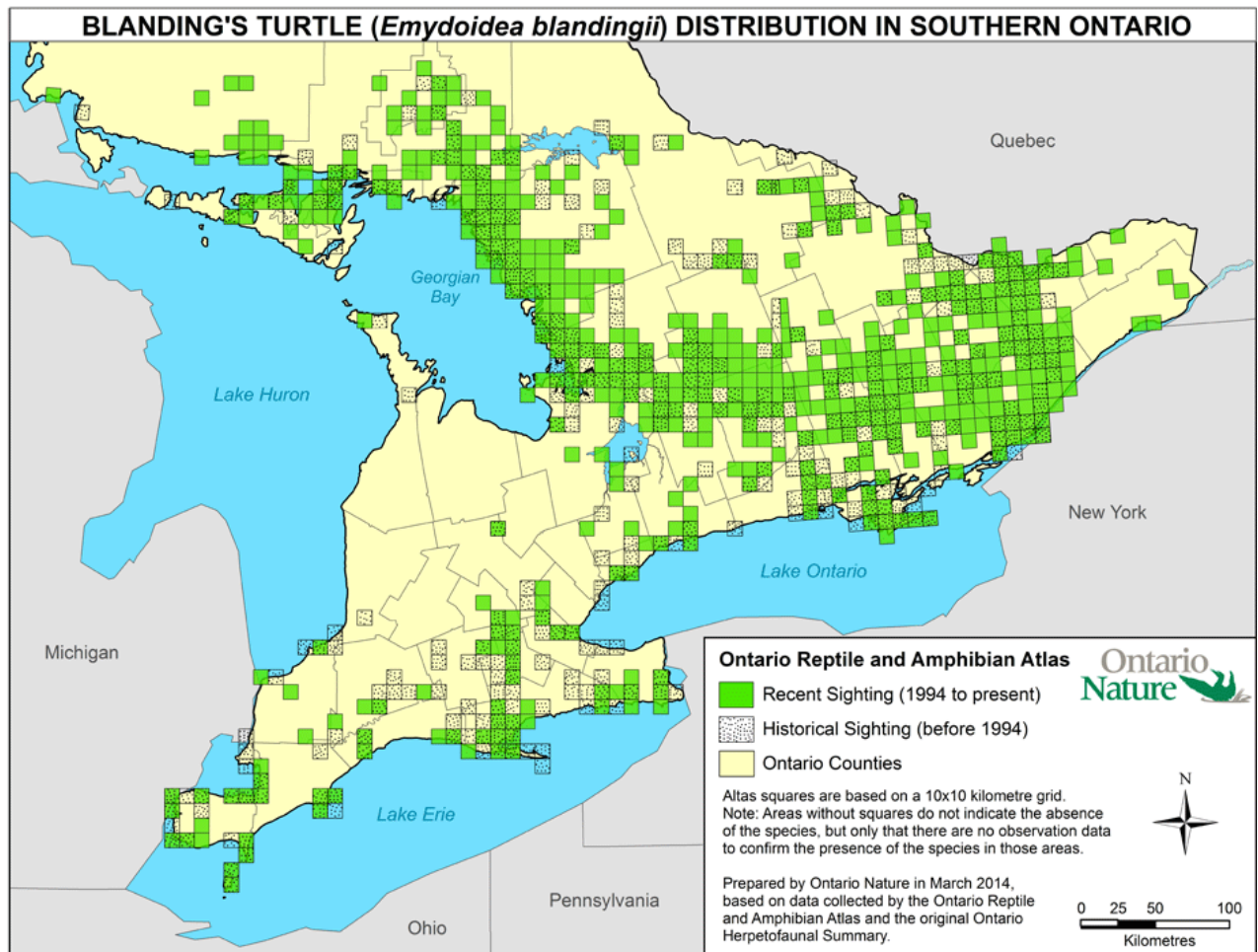
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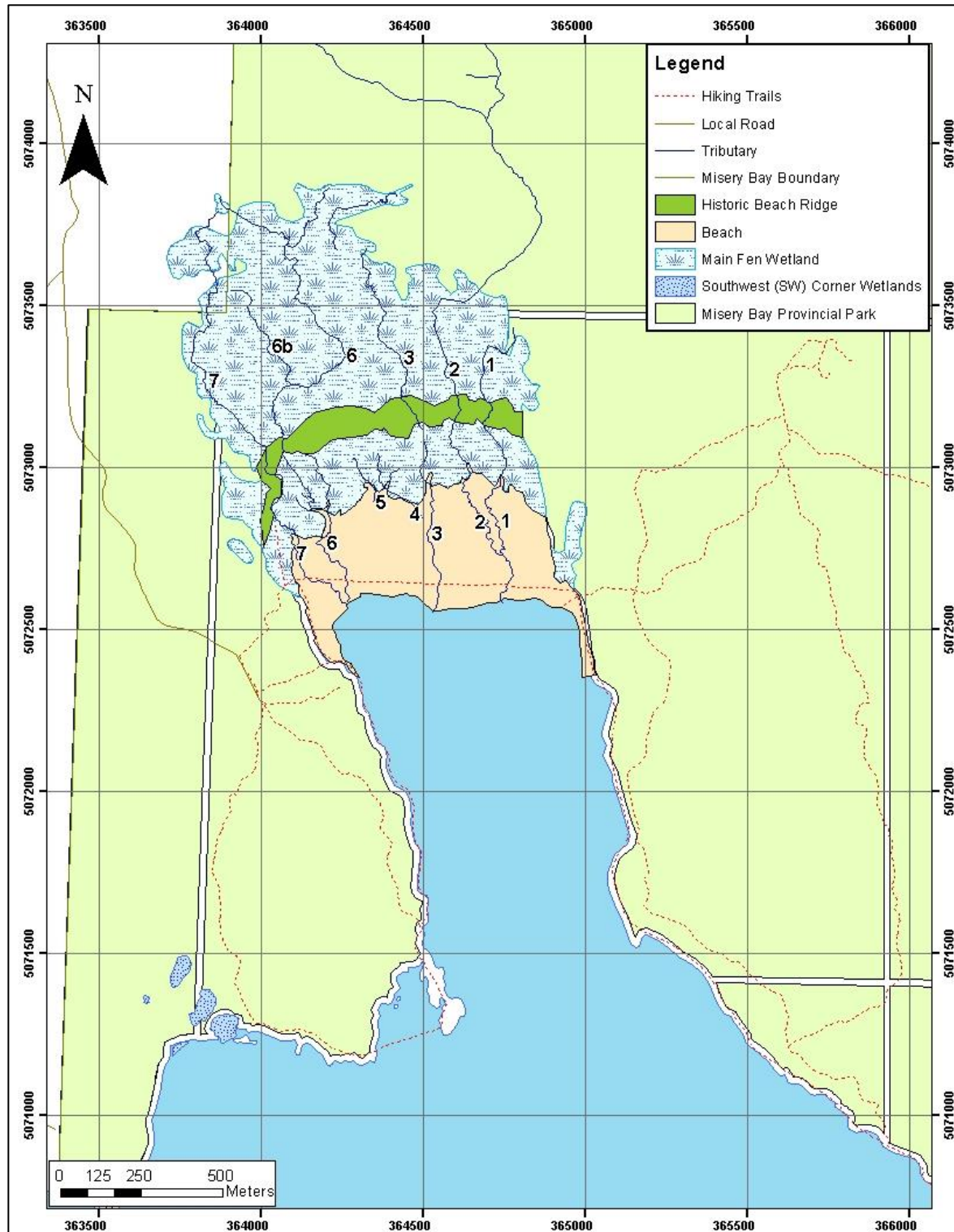
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## Figures



**Figure 0.1:** Ontario range of the Blanding's turtle (*Emydoidea blandingii*) based on historic (before 1994) and recent (1994-present) sighting reports to the Ontario Nature's Reptile and Amphibian Atlas.



**Figure 0.2:** The main wetland areas of Misery Bay Provincial Park, showing the beach area, main fen and southwest (SW) corner wetlands, tributary systems that flow into Misery Bay proper, park trails (subject to dynamic water levels), and local road.

## **Chapter 1:**

**Investigation into the Cause(s) of a mass mortality event of Blanding's turtles in Misery**

**Bay Provincial Park, Ontario, Canada**

## Abstract

Mass mortality events (MMEs) can devastate populations by removing up to 90% of individuals, which is especially damaging in long-lived species. While MMEs are being documented with increased frequency, a limited understanding of the causes and consequences of MMEs remains. My study aimed to determine the causes of a MME of Blanding's turtles (*Emydoidea blandingii*), a threatened species, in a relatively pristine habitat at Misery Bay Provincial Park (MBPP), Ontario, Canada. The typical anthropogenic threats to turtles are minor or virtually absent in the MBPP setting, and yet 53 Blanding's turtles were found dead without obvious cause in 2013. Potential causes of death under consideration included disease, predation in the active season and failed overwintering through either metabolic/respiratory acidosis, freezing, and winter predation. Telemetry and mark-recapture studies were used to determine areas of importance within MBPP and to generate a population size estimate, respectively. Motion-sensor activated trail cameras were paired with Blanding's turtle decoys, as a novel strategy to identify predators within the park. Potential predators identified included the North American river otter (*Lontra canadensis*), mink (*Neovison vison*), coyote (*Canis latrans*), and raccoon (*Procyon lotor*). Overwintering sites were located through telemetry, and temperature and dissolved oxygen content of water were measured to determine differences between known overwintering sites and sites which yielded carcasses, but no significant differences were found. Based on evidence collected, predation as the cause of death has received the most support. The results of my study will be informative for the conservation of the study population, and for the management of future MMEs of turtles in other locations.

## **Introduction**

The causes of mass mortality events (MMEs) of animals are varied, and as such, MMEs must be investigated on a case-by-case basis. Fey et al., (2015) identified the main causes of MMEs to be catastrophic mortality resulting from disease (26.3%), followed by human perturbation through environmental contamination (18.3%), biotoxicity as a result of algal blooms (15.6%), and the collective processes influenced by climate (24.7%). Furthermore, the frequency of MMEs as a result of multiple stressors and disease are increasing (Fey et al., 2015). While MMEs can be examined collectively to determine trends across taxa, each MME must first be evaluated individually to understand the mechanism(s) behind the mortality. In the case of the Blanding's turtles at MBPP, most of the remains were only the plastron and carapace sections of the shell, lacking skulls or small bones associated with limbs, as excessive decomposition had occurred before the carcasses were found. I investigated disease, predation, and failed overwintering as potential causes of this MME.

As reptiles, Blanding's turtles are ectothermic, and thus have a close relationship with their environment, namely to regulate body temperature, which is responsible for many internal processes, and ultimately determines the ability of each turtle to properly function. Similar to many animals in northern climates, Blanding's turtles enter a state of dormancy during the winter months (Edge et al., 2009). Although winter mortality is typically low among freshwater turtles, sporadic events are known to result in high mortality during the winter season, when a depressed metabolic state increases vulnerability of individuals (Ultsch, 2006; Bodie and Semlitsch, 2000; Brooks et al., 1991). The three main threats to overwintering survival are predation, metabolic



and respiratory acidosis, and freezing of body tissues (Edge et al., 2009; Ultsch, 2006), each of which were evaluated as possible causes of death in the Misery Bay MME.

*a) Disease*

Disease was previously examined and determined to be an unlikely cause of the MME, but the tissue samples collected from carcasses may not have been viable for testing (Mendler, 2014). In addition, no turtles displayed symptoms of disease at MBPP in the 2013-15 field seasons.

Disease has not been identified in turtles elsewhere in Ontario (Carstairs pers. comm., 2014), so the spectrum of potential diseases is quite broad and must be narrowed before this hypothesis can be effectively evaluated. Despite these challenges, toe clip samples from 30 adult leopard frogs (*Lithobates pipiens*) were collected in MBPP in 2015 and analyzed for the presence of ranavirus, which has been known to lead to turtle mortality elsewhere (Allender et al., 2011).

*b) Predation (summer and winter seasons)*

While predation is a common cause of death for eggs, hatchlings and juveniles, the risk of predation typically decreases for adult turtles (Standing et al., 1999; Congdon et al., 1994). For example, annual survivorship of adult turtles in a long-term study of Blanding's turtles in the U.S.A. was 96%, compared to 78% for juveniles and subadults, and a mere 26% for hatchlings (Congdon et al. 1993). The absence of a large assembly of literature regarding predation of adult turtles during the active season suggests that the robust nature of a mature turtle shell, paired with their claws and intimidation tactics, are effective predator deterrents. However, rare predation events of adults have been identified as a cause of other MMEs of turtle species in Ontario and elsewhere, in both warm and cold climate conditions (Fincham and Lambrechts,

2014; Stacy et al., 2014; Lanszki et al., 2006; Ultsch, 2006; Brooks et al., 1991), and will be considered temporally throughout my study. Species of the Mustelidae family have been identified as predators in previous chelonian MMEs (Stacy et al., 2014; Lanszki et al., 2006;).

Lanszki et al. (2006) and Brooks et al. (1991) both describe MMEs in which excessive predation occurred during the winter season. Musetlids, such as mink (*Neovison vison*) or otters (*Lontra canadensis*), would have employed their most frequently utilized foraging strategy, termed patchy fishing, by which these mammals repeatedly dive and search for food in a small area of water (Kruuk and Moorhouse, 1990; Kruuk et al., 1990). During the winter season, turtles are unable to actively defend themselves against predators, as their metabolic states are suppressed and their physical abilities are severely limited by cold temperatures. In the cases presented by both Lanszki et al. (2006) and Brooks et al. (1991), dead turtles were found surrounding streams during the spring. Based on the concentration of carcasses surrounding tributaries at MBPP, it seems reasonable that otters or mink may have been utilizing this foraging strategy during the winter, when turtles were overwintering communally within tributaries of MBPP.

### *c) Metabolic and Respiratory Acidosis*

Turtles are able to survive harsh winter months in the northern limits of their range by depressing their metabolic rates, and thus requirements, and by remaining in aquatic sites that are subjected to relatively stable thermal conditions (Ultsch, 2006; 1989). Overwintering site selection differs among turtle species and likely reflects a species' ability to tolerate anoxic conditions (Edge et al., 2009; Newton and Herman, 2009; Crawford, 1990). Freshwater turtles can be either anoxia-tolerant or anoxia-intolerant, based on the concentration of extracellular bicarbonate ( $\text{HCO}_3^-$ ) and

the ability of the shell and skeletal bones to buffer against detrimental lactic acid buildup in body tissues during anaerobic metabolism (Jackson et al., 2007; Ultsch, 2006; Jackson, 2004). Anoxia-tolerant turtles are able to withstand anoxic conditions by depressing their metabolic rate to ~10% of the aerobic rate at the same temperature (Jackson et al., 2000). Metabolic end products, primarily lactic acid in the case of overwintering turtles, remain within the body during the winter season, and accumulate over several months of dormancy. Much of the carbon dioxide ( $\text{CO}_2$ ) in the body is converted into carbonic acid ( $\text{H}_2\text{CO}_3$ ), which can quickly become bicarbonate ( $\text{HCO}_3^-$ ), a compound particularly effective in the buffering of lactic acid (Jackson et al., 2000). However, excessive amounts of lactic acid require additional buffering to prevent damage to the brain and other tissues. In their metabolically reduced state, the availability and diffusion of oxygen will reduce the concentration of lactic acid and other anaerobic byproducts in extracellular tissues (Bagatto and Henry, 1999).

While the tolerance of Blanding's turtles to anoxic environments has not been confirmed in the lab, in the wild they are able to overwinter in aquatic sites with low dissolved oxygen. These overwintering sites also contain painted (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*), both of which are known to be anoxia-tolerant from lab studies, so it is likely that Blanding's turtles share this trait (Edge et al. 2009; Ultsch, 2006). Furthermore, the species will often move short distances during the winter season, perhaps in an effort to shuttle to areas with higher dissolved oxygen, to improve their physiological condition (Edge et al., 2009; Newton and Herman, 2009; Ultsch, 2006). With several tributaries (numbered 1-7) flowing throughout the MBPP wetlands (Figure 0.2), it is possible that turtles chose overwintering sites that were closer to these streams in an attempt to acquire more oxygen. Factors that limit movement or

access to dissolved oxygen in flowing water, such as ice, excessive woody debris or unusually shallow water, may have caused the Blanding's turtles to exceed their physiological capacities, resulting in death due to insufficient oxygen availability.

#### *d) Freezing*

Quite simply, overwintering adult turtles are not freeze tolerant, and when caught in solid ice are unable to access oxygen, and are also immobilized and unable to relocate themselves to areas with optimal temperature conditions. If they are unable to access temperatures that are slightly above freezing, turtles are at risk of irreversible tissue damage (Ultsch, 2006). Winterkill by freezing has been associated with drying of ponds (Bodie and Semlitsch 2000; Christiansen and Bickham 1989), in situations where the drying occurred such that ice formed from surface to substrate, with an insufficient layer of liquid water in between.

#### *Potential Consequences of a Drought*

Lake Huron experienced record low water levels in 2012 (Gronewold et al., 2013), a potential consequence of global climate change, which would have resulted in the drying of the many coastal wetlands (Burton and Uzarski, 2009). While the MBPP wetland appears to drain into, rather than being supplied by, Lake Huron, it can be inferred that the wetland would have also experienced lower than normal water levels. Unfortunately, no historic water level records have been kept at MBPP, so quantifying a drought specifically at the study site will be difficult.

The Misery Bay wetlands are unusually shallow habitat for Blanding's turtles (Sheppard, 2014a, COSEWIC 2005), and a drought in 2012 may have exacerbated the risk of mortality for this

population (Sheppard, 2014a). Drought conditions would have caused lower than normal water levels in the fall, as turtles retreat to their overwintering sites, increasing the threats involved with failed overwintering, and leading to increased winter mortality. These threats include ice penetration from surface to substrate, with an insufficient layer of water between. This could have effectively trapped turtles either in ice or between barriers of ice, limiting their movement and ability to acquire oxygen. They may have been unable to emerge in spring, as water temperatures rose and caused their metabolic rates to increase as well, depleting stored energy and oxygen. In these cases, carcasses would be expected to be found close to tributaries, particularly in the large MBPP fen, where turtles are now known to overwinter (see Results). It is important to consider that conditions of the wetlands at MBPP may have been altered at the time of the mortality event, such that areas that are seemingly fit for successful Blanding's turtles during the winter months of my study period were previously less favorable. Having no winter habitat data prior to or during the period of mortality, I could not fully address this possibility. Alternatively, if a drought occurred during the active season, turtles may have retreated into the tributaries of the fen to increase their access to water as an alternative to entering Misery Bay proper, which has little vegetation available for cryptic basking and as habitat for food sources. Such congregation of Blanding's turtles in narrow tributaries may have made them relatively easy for predators to locate and exploit.

### *Objective, Hypotheses, and Predictions*

The objective of my study was to determine the cause(s) of the mass mortality event of Blanding's turtles at Misery Bay Provincial Park, Ontario. More specifically, I attempted to provide evidence to narrow the speculated causes of death. The theory of multiple working

hypotheses was employed to identify the factors that led to this unusually high mortality (Chamberlin, 1890). Several hypotheses were evaluated, keeping in mind that they may not be mutually exclusive or exhaustive.

I hypothesized that the mass mortality of Blanding's turtles in MBPP was caused by one or more factors, including disease, predation and unsuccessful overwintering. If disease was the cause of the mortality in MBPP, then I expected to find live turtles exhibiting symptoms of disease during the study. Additionally, other organisms in MBPP may exhibit symptoms of disease, including fish or amphibians, which can transmit some disease between themselves and turtles. If predation was the cause of mortality in MBPP, then predators of Blanding's turtles should be present within the park, and there should be evidence that either successful predation and/or attempted predation have occurred, such as additional carcasses found, claw/teeth marks on turtle shells, photos of known turtle predators captured on wildlife cameras, and/or destroyed turtle decoys. If predation was isolated to the winter season, then evidence such as scat, feeding holes, tracks in snow, and wildlife camera photographs that indicate the presence of predators should be present during the winter in areas that turtles use for overwintering. If overwintering failure is the cause of the mortality event in MBPP, then dissolved oxygen content in the water will differ between areas where turtles are known to overwinter and areas where no turtles are known to overwinter. If the Blanding's turtles died due to acidic conditions, then insufficient dissolved oxygen levels will be detected in the water of areas where carcasses were found. In addition, water temperature will differ between areas where turtles are known to overwinter and areas where no turtles are known to overwinter. If winter temperature was the cause of mortality at MBPP, then temperatures will be below freezing in areas of mortality, or ice penetration will occur from

surface to substrate in these areas, leaving an inadequate layer of water between these two media. Again, it is crucial to consider that natural habitats are dynamic, and that the conditions observed through the duration of this study may not be representative of the conditions that were experienced during the mortality event.

## **Methods**

### *Study Site*

My study focused on two main areas within MBPP (Figure 0.2). The “main fen” area is north of Misery Bay proper and includes two large wetlands (~90 ha in total), separated by a historic beach ridge, which runs east-west and is identified by the surplus of substrate and large trees growing along it, effectively dividing the two wetlands. The north fen areas are saturated with water, and are deepest in several pools throughout the area north of the beach ridge, as well as in the seven tributaries that run in a north-south direction through both fens (Figure 0.2). Park trails avoid these wetland areas, as they are difficult to access without proper equipment and are very sensitive to damage done by excessive trampling. The turtle carcasses were found in this north fen area, primarily associated with the tributaries.

The secondary area, the “southwest (SW) corner” includes several small wetlands/vernal ponds to the west of Misery Bay proper (Figure 0.2), where Blanding’s turtles have also been found, but where no carcasses were found. Of these wetlands, one is fen-like in plant and substrate characteristics, and the others are largely situated on depressions of alvar rock where water accumulates in the spring and early summer. Most of these dry up as the summer season progresses, but a few are directly supplied by overflow from Lake Huron, and contain at least 50 cm of water throughout the summer season. Park trails avoid two of these wetlands, which are

concealed within the forest, but trails do come in close proximity of one vernal pond and two coastal pools that are used by the turtles in the summer months.

#### *Time of Mass Mortality Event*

The season in which this MME occurred was uncertain, as the carcasses were found after significant decomposition had occurred, indicating that time had passed since the mortality event. Knowledge of the decomposition rate of Blanding's turtles, or species with similar anatomy, would enable a timeline to be constructed, to better estimate the decomposition rate of freshwater turtle carcasses in this habitat, and thus infer the approximate timing of the MME in MBPP. A freshly dead painted turtle was found in the main fen wetland on 11 May 2014, just outside of the MBPP borders, and photographed periodically from this date to 18 August 2014. It was left without any alterations or caging (as would be done to keep predators from accessing the carcass) at the study site, paired with a wildlife camera, and regularly visited by researchers to monitor decomposition and to identify any scavengers. The decomposition that occurred was a result of climate, such as temperature and humidity, and also of scavengers, including invertebrates. Additionally, one Blanding's turtle was found dead on a nearby road, presumably after being struck by a vehicle, in July 2015. The carcass was paired with a wildlife camera, and was placed in the main fen area of MBPP. It was also periodically photographed to monitor rate of decay. Literature regarding decay rates of hard-shelled turtles was also consulted to determine an estimated time of death.



### *Haphazard Surveys and Telemetry Studies*

Mark-recapture surveys and radio telemetry studies were carried out during the 2014 and 2015 field seasons. Researchers began surveying for turtles on 4 May 2014, and on 2 May 2015, and haphazard surveys were employed to find Blanding's turtles in fens, specifically focused on tributaries and known overwintering sites, and in flooded vernal pools. Wetlands were searched on foot, and turtles were caught by hand, processed (i.e., marked, measured, weighed), and released at the site of capture (Table 1.1). In the first year of study (2013), 30 Blanding's turtles were captured for the first time and marked by Ecologists from Ontario Parks. In the 2014 season, a total of 38 Blanding's turtles was captured, 20 new and 18 recaptured individuals, (18 females, 17 males, and 3 juveniles/subadults). In the 2015 field season, 41 Blanding's turtles were captured, 15 new and 26 recaptured individuals (16 females, 18 males, and 7 juveniles/subadults; Figure 1.1). Upon capture, each turtle was marked using a unique set of identification notches on the marginal scutes (Cagle 1939). Haphazard searches were emphasized prior to July of each year, as turtles became increasingly difficult to find as the summer progressed.

Eighteen Blanding's turtles ( $N_{\text{male}} = 10$ ,  $N_{\text{female}} = 8$ ) were equipped with Holohil RI-2B transmitters (Holohil Systems Ltd., Carp, Ontario) in May and June of 2014. Six of these 18 had been part of the preliminary telemetry study of 2013 (Sheppard, 2014a, b). Twelve of these turtles were initially captured in the main fen area, while the other six were located in the SW corner area of the park (Figure 0.2). Seven additional turtles were added to the telemetry study in 2015 ( $N_{\text{male}} = 5$ ,  $N_{\text{female}} = 2$ ). No gender preference was given to the turtles selected for the telemetry study, as the mortality did not appear to have been selective of one sex over the other

( $N_{\text{dead total}} = 59$ ,  $N_{\text{males}} = 13$ ,  $N_{\text{females}} = 20$ ,  $N_{\text{unknown}} = 26$ ). With few exceptions, radio-tagged turtles were tracked a minimum of twice per week, and location (recorded with a handheld GPSMAP 64s unit, Garmin International Inc., Olathe, Kansas, USA), behaviour, and habitat data were collected, with increased tracking of gravid females during the nesting season (June-July, 2014 and 2015). Radio-tagged turtles were located with a receiver (R-1000 Receiver, Communications Specialists Inc., Orange, California, USA; R410 Scanning Receiver, Advanced Telemetry Systems, Isanti, Minnesota, USA) and 3-element Yagi antenna.

### *Detection of Disease*

The nature of the MME, in which many individuals died in one wetland in a short period of time, is consistent with accounts of reptiles infected by disease elsewhere (Allender et al., 2011).

While no live turtles have been found to have symptoms of disease, and the carcasses had too little viable tissues to sufficiently test for disease, it is possible for certain diseases, such as ranavirus, to be transferred between amphibians and turtles (Brenes et al., 2014). Ranavirus has been found to have led to the death of turtle species elsewhere in North America (Allender et al., 2011). For this reason, toe clips were collected from 30 juvenile and adult leopard frogs using sterilized scissors on 6 August 2015 and tested for the presence of ranavirus in amphibians at MBPP via Quantitative Polymerase Chain Reaction (qPCR). Toe clips were stored in ethanol and frozen until processing. Deoxyribonucleic acid (DNA) was extracted from each sample following the Qiagen DNeasy Animal Tissue (Spin-Column) protocol. Samples were measured for DNA concentration (ng/microL) and purity (A260/A280 ratio) with a NanoDrop 8000 UV-Vis Spectrophotometer.

The above samples were tested by Samantha A. Grant, M.Sc. Candidate in Conservation and Population Genetics, Department of Biology, Trent University, Ontario, using the subsequent protocol. Samples were tested for Frog Virus 3 by amplifying the Major Capsid protein (MCP). Primers were designed based on sets from Hyatt et al. (2000) and were modified to capture an extension of the MCP, approximately 1,500bp, whereas the MCP is 1,392bp. Primers seqMCP-for (5'-<sup>35</sup>TCCACAGTCACCGTGTATCTT<sup>-15</sup>)(Hyatt et al., 2000) and seqMCP-rev (5'-<sup>1506</sup>TGCAGCAAACGGACACTT<sup>1489</sup>) were designed to targeted only Frog Virus 3 and SSME strains of ranavirus. Samples were amplified using Eppendorf Mastercycler Pro PCR System. Each sample for PCR amplification consisted of 1 X PCR buffer (Tris-HCl 50mM, pH 9.0, NaCl 50mM), dNTP 200uM, MgCl 1.5 mM, BSA 0.15mg/mL, 0.3µM of each primer, 1 U Taq DNA Polymerase (Promega), 4µL template DNA, and distilled water for a final reaction volume of 15µL. Thermocycling conditions consisted of 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min for 38 cycles, followed by a final extension for 2 min at 72°C. Two positives were added, including a 100 PFU (plaque forming units) sample of FV3 cultured in house (POS 100), and extracted liver DNA from a previously tested infected wood frog (*L. sylvaticus*) showing strong signs of ranavirus (POS AMW, Laurentian University). A positive and a PCR negative were run along with unknown controls. Amplified product was then separated by electrophoresis on 1.5% agarose gels at 125 v for 30 min, then visualized with ethidium bromide under ultraviolet light. A summary of initial and final PCR reagent concentrations can be found in Appendix I.I.

### *Predator Identification*

A main objective of the field study was to identify potential predators present within MBPP, specifically in close vicinity to wetlands used by the Blanding's turtles. This initiative was carried out throughout all seasons by four means: i) motion-activated wildlife cameras placed throughout the wetland areas, ii) evaluation of all captured turtles for bite and scratch marks, iii) Blanding's turtle decoys deployed in wetland areas to entice and attract predators to wildlife cameras, and iv) the identification of predators through winter tracking of footprints and scat in the snow.

Three motion-detecting wildlife cameras (Stealth Cam Skout 7MP HD, Grand Prairie, Texas, and two Tasco 119215c, Overland Park, Kansas) were deployed in the park in early May 2014, focusing on the tributary streams that flow through the large fen wetland area. These cameras were removed from the park prior to the 2014-15 winter season. To reduce the risk of camera seizure in extreme cold temperatures, six high performance wildlife cameras (Reconyx Hyperfire™ PC900, Reconyx Inc., Holmen, WI, USA) were later deployed on 25 and 26 January 2015. Each camera was programmed to take a burst of three photographs each time it detected motion, and were capable of taking photos both in daylight and at night. These cameras were set up in wetlands that experienced mortality and those that did not, with an emphasis on the area where mortality occurred. Some of the cameras were periodically relocated to maximize exposure of the wetlands, and to focus on areas where signs of predators, such as tracks, scat, and/or disturbed vegetation, were observed. In addition to cameras being present throughout the winter season, researchers visited MBPP biweekly through September and October of 2014 and 2015, and approximately once per month from November 2014/15 – April 2015/16, conducting

haphazard surveys to identify footprints, other tracks/trails, and feeding holes left in the snow/ice by potential predators. Photo records and GPS waypoints were recorded for tracks of interest. In addition to the wildlife cameras deployed during the active season, all turtles found, either by telemetry or haphazard surveys, were examined for carapace and plastron damage, such as scratches and bite marks that may have suggested failed predation attempts.

Two Blanding's turtle decoys were placed to entice predators so that they could be identified through photos taken by accompanying motion-sensing wildlife cameras. Each decoy was anchored to the ground using a plastic-covered tree-stand wire, to increase the likelihood of multiple pictures being taken of animals that attempted to attack or drag the decoys away. While a turtle decoy has been used previously by Ashley et al. (2007) to determine the incidence of intentional vehicle-reptile collisions, their use to lure and identify natural-occurring predators is novel. The decoys were removed from the field in November 2014 and December 2015, as they would be minimally effective during the winter after being buried by snow. To enhance their appeal to predators before deployment in the 2015 field season, the decoys were bathed in water from captive Blanding's turtles at Science North in Sudbury, Ontario. This aided in eliminating their persistent synthetic odour and provided more naturally smelling turtle decoys at the beginning of the 2015 field season. The two decoys were also doused with excrement from live turtles, which was collected from Blanding's turtles in a standard 5-gallon bucket as they were found and processed during the 2015 field season. These efforts were made to enhance the attractiveness of the decoys to potential predators. Ultimately, photographs, markings on turtle shells, interaction with decoys, and footprints found in the winter season were used to confirm or refute the presence of potential predators in various areas of MBPP.

The pictures from each camera were examined, and counts of all animals photographed were made, including both potential predators and benign species. The location of predatory animals relative to areas where Blanding's turtles were located during various seasons were considered when interpreting the significance of the photographs. Since animals could not be recognized as individuals, each animal was considered a new/unique individual if more than approximately 30 seconds elapsed between sequences of photographs of the same species. The activity and/or direction of travel of the animals was also taken into consideration when deciding whether multiple sequences had captured photographs of the same individual, or if they were multiple different individuals of the same species.

#### *Overwintering Site Characteristics: Temperature*

Characteristics of sites throughout the wetland were recorded beginning in October 2014 and 2015. Many of the radio-tagged turtles (10 individuals in winter 2014/15 and 21 individuals in winter 2015/16) had temperature loggers (iButtons DS1920, Embedded Datasystems, Lawrenceburg, KY, USA) adhered to their shells, which began recording temperature at 3-hour intervals in October 2014 and 2015. They were removed from turtles in the spring of 2015 and 2016, once turtles had emerged from their overwintering sites. Temperature data were obtained from nine overwintering turtles in winter 2014/15 and 16 turtles in winter 2015/16. Eleven bricks, serving as haphazard temperature stations, were outfitted with iButtons and were deployed at sites (haphazard stations) where no turtles are known to have overwintered in 2014/15, and an additional seven (total =18) were deployed in fall 2015, which recorded temperature at the same frequency (every 3 hours), and approximately the same verticle position

in the wetland, as those on turtles' shells. Therefore, the two types of temperature-recording stations used in my study were turtles and haphazard brick temperature stations.

General linear models (GLMs) were used to determine the relationship between water temperature and several fixed effects. The fixed effects included the two measurement station types (turtle and haphazard), areas of the park (main fen and SW corner), mortality or non-mortality sites (determined based on whether at least one carcass was found at the site), and year (winter 2014/15 or winter 2015/16), were examined using GLMs that included a random effect of ID to account for repeated measures. This analysis was conducted in R (R Core Team, 2016) using the nlme (Pinheiro et al., 2016) and car (Fox and Weisberg, 2011) packages. A visual histogram assessment found the temperature variable to be normally distributed, and therefore no data transformation was applied.

#### *Overwintering Site Characteristics: Dissolved Oxygen*

Dissolved oxygen content in the water was measured at several sites in both the main fen and SW corner areas of the park. Due to difficulties in the field, including auger failure and drilling into organic substrate (rather than into a layer of water) at many measuring sites, measurements were inconsistent in the 2014/15 field season, resulting in unusable data for that winter.

Successful dissolved oxygen measurements were obtained during the 2015/16 winter season after more precisely marking the spots at which readings were to be taken before wetlands were covered in ice and snow. As such, the repeated measures ANOVA for dissolved oxygen took into account only data from the 2015/16 winter season. Two Certified® 122-cm reflective fiberglass orange poles were used to mark each measurement site, with the deepest area of water

equidistant from each, so that the point at which the measurement hole was to be made in the ice had a lesser chance of meeting an area without water. Each site was visited once per month at 4-week intervals: 8 and 9 January, 5 and 6 February, and 5 and 6 March 2016. A hole was created in the ice with either an ice pick or a hand auger at locations where turtles are known to be overwintering and at haphazard stations. Dissolved oxygen was measured in mg/L using a handheld probe (Extech DO600-K Waterproof ExStik II Dissolved Oxygen Meter Kit, Melrose, MA, USA) by inserting the probe ~10 cm into the water column, while gently moving it to ensure the continuous flow of water over the membrane of the instrument.

Similar to the analyses described previously, GLMs were used to determine the relationship between the dissolved oxygen content in the water and several fixed effects. The fixed effects included the two measurement station types (turtle and haphazard), areas of the park (main fen and SW corner), and mortality or non-mortality sites (determined based on whether at least one carcass was found at the site), were examined using GLMs that included a random effect of ID to account for repeated measures. This analysis was conducted in R (R Core Team, 2016) using the nlme (Pinheiro et al., 2016) and car (Fox and Weisberg, 2011) packages. Dissolved oxygen content in the water was the dependent variable, and the independent variables were two measurement station types, areas of the park (Figure 0.2), and mortality and non-mortality sites. A Shapiro-Wilk normality test found the dependent variable to be left-skewed. The square of each data point ( $x^2$ ) was calculated to achieve normality.



## Results

### *Time of Mortality: Turtle Carcasses Found in 2012*

The turtle carcasses were discovered dead without obvious cause in the spring/summer of 2013. How long the carcasses were present and unnoticed was not known. The majority of the Blanding's turtle carcasses ( $N = 53$ ) were found in close proximity to tributaries, which run north-south through the fen, eventually flowing into Misery Bay proper. Two photographs (Plate 1.1a, b), taken by L. Reid on 18 July 2012, indicate that this turtle was likely dead for less than two weeks when photographed, based on the detailed decomposition monitoring of two freshwater turtles in MBPP (see below). All of the limbs and tail were intact, and showed no sign of desiccation in the photographs. The head was described as being "gnawed off," and this would likely not have been so obvious if the carcass had significantly progressed through the stages of decomposition. Reid (pers. comm., 2016) recalls that she saw two dead turtles that day, about 10 m apart in the wetland, but only photographed one. The shell of the un-photographed turtle was described as being dried and scattered. Reid also noted that it was a very hot and dry summer, during which plants bloomed earlier than in previous years.

Additionally, two Blanding's turtle carcasses were found by a park visitor in August 2012 on the west side of the park, in the wetland between Misery Bay proper and the historical beach ridge/treeline (Figure 0.2; McFadden, pers. comm., 2016.). McFadden described the water level as being lower than it had previously been at MBPP. While carcasses showed slight evidence of decomposition, one of the carcasses had no marks on the shell, limbs, or tail, but the neck had been torn open and was still attached to the head and the body (McFadden, pers. comm., 2016). A second carcass was located approximately 80 m from the first, and was described as missing

its head and one of its limbs (McFadden, pers. comm., 2016). One painted turtle was found by a third park visitor (name unknown) in the late summer of 2012 and is now desiccated and used as a display specimen in the MBPP Visitor's Center (Sheppard 2014a).

#### *Time of Mortality – Decomposition Monitoring*

The painted turtle carcass found on 11 May 2014 appeared to be freshly dead. The appendages, including limbs, head, and tail, were intact and only the limbs showed some shriveling at the distal ends. By 22 May 2014, the decomposition process had progressed, evident by shriveling at the ends of extremities and a sunken head, but the viscera remained enclosed within tissue; no holes were present (Plate 1.2a-f). By 4 June 2014, the limb, head, and tail tissue had decomposed significantly, such that one could see through the space between the plastron and carapace, looking from anterior through to the posterior, with only some obstruction in the line of sight (Plate 1.2g, h). On 26 June 2014, only the shell and bones remained; there was no soft tissue remaining on the carcass (Plate 1.2i, j). Thus, in spring/summer conditions, it appears that carcasses take approximately 3 weeks to significantly decompose under normal weather condition (average temperature June 2012-July 2013 = 15.4°C, average precipitation June 2012-July 2013 = 2.5mm; Environment Canada: <http://climate.weather.gc.ca>), and 5-6 weeks to decompose to the point at which no soft tissue remains. The carapace and plastron remained attached via the bridge scutes at this point, with the sutures between scutes remaining strong.

The roadkill Blanding's turtle that had been eviscerated prior to being placed in the wetland on 26 June 2015 (Plate 1.3) did not allow for the full rate of decay to be observed. It was clear that exposed tissue subjected to drying in the summer weather became tough and was not stripped

from the carcass, even after several weeks (Plate 1.3d). The tissue in areas which were submerged in water had decomposed to the point at which the boney elements appeared clean (Plate 1.3e). This carcass was not collected after the summer field season, but was found to be missing on 8 January 2015. Examination of the motion-activated wildlife camera that had been paired with the carcass showed a coyote approach and smell the carcass. While no photos were taken as the coyote left the field of view, the turtle carcass is not visible in the next series of three photos. The carcass was found and photographed during surveys by an Ontario Parks Planner on 1 May 2016, approximately 15 m from where it had been set in front of the wildlife camera. While the carcass had been eviscerated prior to stationing in the wetland, some soft tissue remained hydrated in pieces of the shell that were found in the water (Plate 1.3g). In mid-summer conditions, the portions of the carcass that decomposed the most were those submerged in water. The portions of the carcass that had been desiccated prior to being placed in the wetland, such as the skin of the neck and limbs, did not significantly decompose over the course of the summer and fall months. Through observing the decomposition of this carcass, and comparing it to the painted turtle carcass that was situated in a wetland since its death, it is clear that moisture plays a large role in determining the decomposition rate of freshwater turtles.

#### *Evaluation of Predation as a Cause of Death*

Photos identified the presence of raccoon (*Procyon lotor*), ravens or crows (*Corvus corax* or *Corvus brachyrhyncho*, respectively), coyotes (*Canis latrans*), and river otters (*Lontra canadensis*) in MBPP, all of which can predate turtles (Appendix I.II; Plate 1.4a-d). Numerous other animals were identified through the use of the trail cameras, but are not known to prey on turtles. Additionally, I observed and photographed an eastern mink (*Neovision vision vision*) in

July 2014, on the west side of the Misery Bay coast, within 100 m of Lake Huron proper. An assumed mink trail, with scat, was observed in the north fen area in October 2014, and a trail camera was set up in close proximity in hopes of capturing photos to definitively show their presence, and perhaps capture some behaviour. No mink photos were captured, and the trail was not present in fall 2015.

On 9 December 2015, the decoy in the area where significant mortality had occurred was found about 20 m from where it had been anchored into the fen. Its head and limbs had been removed, likely torn/chewed off by an animal, and were in pieces surrounding the shell of the decoy. Parts that would have contained accessible flesh were targeted and removed, while the carapace and plastron of the decoy remained intact with some minor piercing present, but no tearing. This meticulous pattern of destruction, which is similar to those of carcasses found (very few skulls and limb bones found, plastron and carapace without obvious signs, such as excessive teeth or claw marks, of predation; Mendler, 2014), suggests the decoy was perceived as a turtle, rather than it being used as a play object and destroyed without precision or intent.

One radio-tracked Blanding's turtle experienced what seems to have been a failed predation attempt in September 2014, which left damage to the keratin layer of the posterior area of her shell (Plate 1.5). The turtle showed no signs of serious injury to her limbs, head, or tail, but it was clear that an aggressive attempt had been made to chew on her carapace, near where the transmitter was adhered. Each of these described events suggest that predation is a risk to the Blanding's turtles of MBPP in the active season.

### *Temperature Analysis*

Temperature of overwintering turtles and bricks at haphazard stations were recorded and analyzed for the 2014-2015 and 2015-2016 winter seasons. There was a significant difference in average temperature between the overwintering turtles and the haphazard stations ( $t_{35} = -2.12$ ,  $p < 0.05$ ; Figure 1.2). Average turtle temperature, as measured by dataloggers adhered to the carapace, was  $2.0^{\circ}\text{C}$ , which is  $0.74^{\circ}\text{C}$  lower than the average temperature of the haphazard stations. The haphazard stations recorded lower minimum temperatures, with the lowest temperature recording of  $-5.4^{\circ}\text{C}$  on 27 February 2015, than that of the turtles, which did not record temperatures below  $0^{\circ}\text{C}$ . The highest recorded spring temperatures were those of turtles who reached a maximum temperature of  $19^{\circ}\text{C}$  on 15 March 2016. Additionally, a significant difference in temperature was observed between the two years of study, in which 2015 experienced higher temperatures than 2014 ( $t_1 = 71.88$ ,  $p < 0.0001$ ).

### *Dissolved Oxygen Analysis*

Dissolved oxygen was relatively high and consistent among wetlands at MBPP during the winter of 2015-2016. Average dissolved oxygen was  $13.58 \pm 1.53$  mg/L, maximum was 16.27 mg/L and minimum was 9.13 mg/L. Both the maximum and minimum values were recorded at sites on the east side of the main fen. During the 2016 winter, there were no significant differences in the amount of oxygen present in the water in sites where turtles are now known to overwinter and where no turtles are known to overwinter ( $t_{16} = 0.07$ ,  $p = 0.95$ ). There was no significant difference between dissolved oxygen content of the water at sites where carcasses were found and where no carcasses were found ( $t_{16} = 0.28$ ,  $p = 0.79$ ). No significant difference was found in

dissolved oxygen content of the water between the main fen sites and the SW corner sites ( $t_{16} = 0.83$ ,  $p = 0.42$ ).

#### *Disease: Ranavirus*

None of the 30 leopard frog (*Lithobates pipiens*) samples contained DNA that tested positive for FV3-like virus (Figure 1.3).

### **Discussion**

#### *Decomposition Rate of Freshwater Turtles and Time of Mortality*

While many carcasses were recovered and analyzed for evidence to explain the timing and cause(s) of death, they were so far decomposed at the time of discovery that many clues were no longer apparent. The strongest piece of evidence to suggest the time of mortality are the photos of a dead Blanding's turtle and associated notes submitted by L. Reid, the accounts of J. McFadden of freshly dead Blanding's turtles at MBPP, and the freshly dead painted turtle carcass that was found by another park visitor, all from events in summer 2012. As animals with high adult survivorship, it is unusual to encounter multiple dead adult turtles in a short amount of time (Brooks et al., 1991). Thus, it is reasonable to conclude that the individuals described by these park visitors died during the mass mortality event.

The pattern of decomposition found through the observation of recently dead painted turtle and Blanding's turtle carcasses are consistent with those of Dodd (1995). In the Dodd (1995) study, 80 turtles of three families, including species in the family Emydidae, were laid on sandy substrate and allowed to decompose for up to 54 months in Northern Florida. Based on his 9-stage decomposition classification scheme, the carcasses found at MBPP were between

decomposition stages 4 and 6, whereby the scutes had loosened and begun to peel away from underlying bone, and sutures between scutes were in various stages of separation (Dodd, 1995). Those specimens had reached stage 6 after approximately three months and the author explained that once stage 6 is reached, it lasted for a longer amount of time than the earlier stages, taking an average of 6.3 months for Emydidae turtles to progress to decomposition stage 7, during which bones become disarticulated but are still in close proximity to the carcass (Dodd, 1995). The severity of Ontario winter seasons, which was not experienced by the Dodd (1995) specimens, would have slowed the rate of decay such that approximately eight months of Ontario's seasonal conditions would be required for carcasses to reach stage 7. Through these lines of evidence, park visitor accounts of carcasses found in July and August of 2012, and the decomposition stages/timeline developed by Dodd (1995), the time of the MBPP mortality event is estimated to have been primarily in the months of July and August, 2012. This is further supported through the comparison of locations of remaining live Blanding's turtles, gained through radio telemetry, and the location of carcasses, as the locations of live Blanding's turtles during the 2013, 2014, and 2015 active seasons overlap with the location of carcasses in the months of May-August, but most notably in June-August (Appendix I.III). This shows that the areas where mortality occurred are also areas where Blanding's turtles are found throughout the active season.

#### *Predation as a Potential Cause of Death*

Several chelonian predators are now known to currently reside in, or occasionally frequent MBPP, including raccoons, corvids, coyotes, mink, and river otters. While it was expected that predators would be present in a natural habitat such as this, the confirmation of their current

presence is of importance when evaluating predation as a potential cause of turtle death.

The greatest predator of all life stages of turtles is raccoons, but the majority of the literature identifies them primarily as nest predators (Browne and Hecnar, 2007; Lanszki et al., 2006; Congdon et al., 1983). Predation by raccoons is especially damaging to turtle populations in close proximity to human development, especially in areas where large carnivores have been extirpated (Bennett and Litzgus, 2014; Riley and Litzgus, 2013; COSEWIC, 2005; Rogers and Caro, 1998; Garrott et al., 1993). While raccoons were identified by the wildlife cameras, MBPP is not an area that should be experiencing particularly high predation rates by raccoons, subsidized predation does not occur in this remote, day use-only (no camping permitted) Provincial Park, and large carnivores, namely coyotes, are abundant. Based on the wildlife camera findings, raccoons were present mostly along the beach area in the SW corner and along edges of the main fen, but did venture into the main fen in spring 2016 (Plate 1.4a). In another study of turtle predation, Stacy et al. (2014) stated that raccoons would leave injuries on turtles similar to those of otters. In examining the case at MBPP, it is unlikely that raccoons were responsible for the mortality, but they cannot be ruled out with the evidence available.

Corvids, such as crows and ravens, are additional known predators of adult turtles and tortoises, as they have been found to peck into the abdomen above the tail region of the body tissue, leaving the shell intact and without obvious signs of damage (McCullum 2015; Baxter-Gilbert et al., 2013). Corvids were responsible for the death of 45 wood turtles between 2011 and 2015 at Base Gagetown, New Brunswick, Canada (McCullum 2015). The injuries noted on recently deceased and injured but alive turtles included holes in the body cavity near the legs, and evisceration, but did not include the removal of head and limbs from the carcass (McCullum,



2015). Injured turtles were found in close proximity to a raven or a crow, and a decoy was approached by a raven several times within 26 hours of placement in the field (McCullum, 2015). Both crows and ravens are known to be present within MBPP, identified through the use of wildlife cameras and through frequent sightings while conducting fieldwork within the park. However, without finding the carcasses soon after death, any marks that may have been left on the soft tissue of the turtle carcasses by corvids would have essentially been erased through decomposition. Additionally, the accounts by L. Reid and J. McFadden do not indicate typical signs of corvid predation, and corvids were not found to be particularly interested in either of the two decoys stationed in the park. As such, there is no evidence to suggest that corvids were responsible for the mass mortality event.

Little literature regarding predation of adult turtles by coyotes exists, suggesting that such events are rare. However, the wildlife cameras found that coyotes frequent MBPP main wetland, specifically during the winter season, and in areas where turtles are known to overwinter. Furthermore, one trail camera captured images of a coyote showing interest, through approaching and smelling, in the roadkill turtle that had been relocated to the area where the MME had occurred. The carcass was found on 1 May 2016 during spring surveys, and had only been removed about 15 m from the location where it was stationed. The high availability of common prey species of coyotes, including small mammals, fruit, birds, rabbits, white tailed deer (*Odocoileus virginianus*) on Manitoulin Island may promote coyote success and proliferation to the point at which secondary food sources, such as turtles, must be relied upon. Minckley (1966) reports witnessing a coyote preying upon multiple adult freshwater turtles, in which the coyote caused significant damage to the carapace and plastron of the turtles, and the turtles were found

scattered along a wetland edge and closely associated with coyote scat and tracks. The MBPP turtles were found in proximity to water, but were not associated with scat or tracks. Many adult Blanding's turtles at MBPP exhibit marks on their shells that were likely made during unsuccessful predation attempts, but the turtle carcasses did not exhibit the extensive shell damage (pers. observ.; Mendler 2014) described by Minckley (1966). As such, it is unlikely that they were killed by coyotes. In a report prepared for the U.S. Fish and Wildlife Service, Cypher et al. (2014) outline the methods and results of a study conducted in the central Mojave Desert of California to determine the threat of coyotes to desert tortoises (*Gopherus agassizii*). Based on scat samples, it was determined that desert tortoises are typically secondary food sources to coyotes, but that coyotes will opportunistically prey on desert tortoises (Cypher et al., 2014). When considering the paw anatomy, namely the absence of opposable thumbs required for dexterous manipulation, and the described predation tactics of a coyote, they are ultimately not capable of the fine motor skills necessary to prey upon a turtle without leaving distinctive damage (Cypher et al., 2014; Minckley, 1966). As such, it is clear that predation by coyote was not the cause of the MBPP MME.

Both the North American river otter and American mink have been confirmed to be present within MBPP through photographs, tracks, and fish feeding remains. As a well-known predator of turtles, there is much literature pertaining to turtle predation events by otters (Fincham and Lambrechts, 2014; Stacey et al., 2014; Lanszki et al., 2006; Brooks et al., 1991). The location of carcasses surrounding tributary streams in MBPP where Blanding's turtles are known to overwinter supports the hypothesis that the predation event may have taken place during the winter, and is very similar to conditions noted by both Lanszki et al. (2006) and Brooks et al.

(2001). However, the accounts by park visitors of dead turtles in the summer of 2012 (Plate 1.1), a predation attempt on a Blanding's turtle in fall 2014 (Plate 1.5) and none during the 2014/15 or 2015/16 winter seasons, and the investigation and decomposition timeline by Dodd (1995) all suggest that the MBPP mortality event occurred prior to the winter season.

The home range size and social tendencies of American river otters also suggest that otters are likely the cause of this MME. River otters tend to form groups, either to aid in raising of pups and/or to cooperatively forage (Goreman et al. 2006). By contrast, other mustelids, including American mink, defend intra-sexual territories, possibly to limit the accessibility of resources by other individuals (Yamaguchi and Macdonald, 2003). Cooperative foraging may be beneficial in situations where food resources are not limiting, but are patchily distributed (Macdonald, 1983). This would appear to have been the case in MBPP, where the abundance of Blanding's turtles was high, and their presence was concentrated, at least in part, to the tributaries of the large fen. Goreman et al. (2006) found that the home range size of female otters was 9.56 km<sup>2</sup> and that of males was 30.38 km<sup>2</sup>. While no otters were seen or photographed within the fen where the carcasses were found, these home range sizes indicate that otters from elsewhere in the park could have used that wetland to forage in the summer of 2012. In addition, otters are known to shift parts of their home ranges from year to year in response to changing resource availability (Goreman et al. 2006). It is possible that low water levels in 2012 elicited a change in food availability, leading to a shift from primary to secondary food sources, such as Blanding's turtles, and also a shift in home range size to acquire these secondary food resources (Goreman et al. 2006; Lanszki et al., 2006). Low water levels would have also increased the vulnerability of the Blanding's turtles by confining them to the narrow tributaries of the main fen wetland, which

contained water, while the areas between tributaries that are typically saturated did not contain water (Sheppard, 2014a). Conversely, a shift in otter home range area may have occurred due to the increased presence of researchers in MBPP over the summers of 2013, 2014, and 2015.

The two Blanding's turtle decoys provide further evidence of otter predation. Neither decoy drew the attention of predators during the 2014 field season. In 2015, when the decoys were treated with turtle housing water and excrement, one of them was removed to about 20 m from its stationed location, and had its head and limbs torn off and scattered around the main body. Interestingly, this was in a very similar fashion to the way that the dead Blanding's turtles were found in 2013, whereby the leg, neck, tail, and skull bones (or foam pieces, in the case of the decoy) were missing in most cases. Unfortunately, the wildlife camera did not capture any photos of this event, but the findings suggest that it occurred in a methodical way, providing support for the predation hypothesis. Lanszki et al. (2006) examined the carcasses of 182 European pond turtles (*Emys orbicularis*) that had been preyed upon by otters, and found that in 98% of the cases the head had been consumed, followed by the forelimbs the tail and the hindlimbs in this sequential order (Lanszki et al., 2006). These findings are consistent with the state of the decoy remains, and also with the accounts of dead turtles provided by Reid and McFadden. Despite the wildlife camera not capturing any photos, this evidence strongly suggests a dexterous and non-random deconstruction of the decoy.

Additionally, a study conducted by Stacy et al. (2014) investigated a large scale predation event, resulting in 76 carcasses of two species, Florida cooter (*Pseudemys floridana*) and Florida softshell turtle (*Apalone ferox*), in north central Florida, USA. It was found that otters at these

sites may kill more turtles than they actually consume, as evident by researchers finding multiple live but fatally injured turtles (Stacy et al., 2014). Based on the evidence and information available, there is strong support for otters being the predators responsible for the mysterious turtle deaths at MBPP.

### *Temperature as a Potential Cause of Death*

Turtle temperatures were higher than those of bricks at haphazard stations in the spring of each year, and did not drop below 0°C during the winter, but several haphazard stations experienced temperatures below 0°C. These findings were expected, as it is detrimental to a turtle if body temperature falls below 0°C (Ultsch, 2006). It is possible that Blanding's turtles will make vertical movements in the water column to maintain body temperatures above freezing during the winter (Edge et al., 2009; Bradford, 1983). However, the wetlands of MBPP are relatively shallow, not exceeding about 1.5 m depth at haphazard and turtle overwintering stations. There was no evidence in the findings to suggest that unsuitable water temperature during the winter months was responsible for the MBPP MME.

Interestingly, there was a significant difference between the temperatures recorded during the 2014/15 and 2015/16 winter seasons. When considered with the late onset of ice in the 2015/16 winter season, these results indicate some variability in habitat between years. While the lack of winter data prior to 2014 does not allow quantitative comparisons between the study winters and the 2012/13 winter, the winter following the mortality event, it is clear that water levels and temperature regime vary from year to year; these wetlands are dynamic. It is possible that the 2012/13 winter saw changes in wetland temperature, which could have been a factor that

contributed to the increased mortality. However, the data collected and analyzed in my study do not suggest that the mortality event occurred during, or immediately following, the winter season, or that the temperature conditions of overwintering turtles during the winter season were outside of their survivable range.

#### *Insufficient Dissolved Oxygen as a Potential Cause of Death*

Sites at MBPP in which Blanding's turtles overwintered did not exhibit higher levels of dissolved oxygen than haphazard sites throughout the wetlands, including the areas in which carcasses were found. Similar to the study performed by Edge et al. (2009), I found that Blanding's turtles at MBPP did not select overwintering sites with significantly higher oxygen content than other areas of the wetland. The wetlands of MBPP exhibited ubiquitously high dissolved oxygen (Table 1.2), all of which were much higher than values recorded by Edge et al. (2009). These data suggest that dissolved oxygen of the water was not the cause of the MME at MBPP. However, I cannot assume that the conditions of the wetland during my study were the same as at the time of the MME, especially when acknowledging that record-low water levels were seen in Lake Huron in 2012 (Gronewold et al., 2013). It is possible that an altered water table could have changed the dynamics of the springs that feed water into the main wetland at MBPP (Burton and Uzarski, 2009), altering the amount of dissolved oxygen that reached the overwintering sites of Blanding's turtles within the park. The evidence collected during my study, which shows comparatively high amounts of dissolved oxygen present throughout the wetlands of MBPP, suggests that the oxygen content in the water was sufficient to sustain the turtles through the winter season. However, it is important to consider that the dissolved oxygen content in the water at MBPP prior to 2014 was not available for this study, and may have varied

from the results presented herein, as a result of low water levels.

### *Disease as a Potential Cause of Death*

None of the Leopard frog toe-clip samples tested positive for ranavirus, suggesting that the pathogen is not present in MBPP, and thus could not have been transmitted to the turtles that experienced mortality. While it is possible that the juvenile frogs sampled had not yet encountered the disease in their environment, the absence of live turtles exhibiting symptoms, such as nasal/ocular discharge and oral/cloacal lesions (Allender, 2011), suggests that ranavirus is not currently present in the MBPP Blanding's turtles. Amphibians, infected with ranavirus have been shown to exhibit high mortality rates, progressing quickly to death. This pattern eradicates both infected animals and the pathogen from an area in a short time (Johnson et al., 2007). If this pattern also applies to chelonians, the detection of ranavirus in a population may be dependent on early detection (Allender, 2011). With the data available, there is no evidence to suggest that ranavirus was the cause of the 2012 MME in MBPP. Should ranavirus be found in the Blanding's turtles in MBPP in the future, patterns of mortality should be compared with those presented in my study. Finally, it is important to state that reptile-pathogen interactions are not well known, and there are likely many undiscovered diseases that affect these animals.

### *Potential Role of Drought in the Misery Bay Blanding's Turtle Mortality*

Large scale mortality of chelonians coinciding with unusual environmental conditions, specifically drought, have been well documented (Fey et al., 2015; Cypher et al., 2014; Stacy et al., 2014; Anthonysamy et al., 2013; Rowe et al., 2013; COSEWIC, 2005; Aresco et al., 2003; Longshore et al., 2003; Reed et al., 2003; Hall and Cuthbert, 2000; Christiansen and Bickham,

1989; Ultsch, 1989; Gibbons et al, 1983). As a semi-aquatic species, Blanding's turtles are found in association with wetlands including lakes, ponds, fens, and bogs (Edge et al., 2009; COSEWIC 2005), and are dependent on the condition of these water bodies. Droughts exacerbate the challenges that are already faced by Blanding's turtles in their natural habitats, increasing stress and susceptibility to winterkill, predation, disease, and road mortality (Hall and Cuthbert, 2000; Christiansen and Bickham, 1989; Ultsch, 1989). Low water levels in the fall can have devastating effects on turtles throughout the winter season, as a sufficient layer of water may not remain between the ice and substrate (Hall and Cuthbert, 2000; Ultsch, 1989). In the active season, low water levels can make areas of wetlands more accessible to predators, and can also concentrate turtles into areas where water remains, increasing their vulnerability to predation (Stacy et al., 2014; Hall and Cuthbert, 2000). Low water levels could have also concentrated the turtles into areas where water remained, increasing their proximity to one another and potentially increasing their susceptibility to disease transmission (Allender, 2011). Altering properties of wetlands also has an effect on the movement patterns of the turtles that inhabit them, as individuals may be forced to travel across land to find other suitable habitat (COSEWIC, 2005; Aresco et al., 2003; Hall and Cuthbert, 2000; Gibbons et al., 1983).

Hall and Cuthbert (2000) compared mortality of Blanding's turtles in Minnesota at two sites, one that experienced a planned wetland drawdown (simulating drought conditions) to create waterfowl habitat, and a site that did not experience a drawdown. The drawdown site experienced 50% turtle mortality, while no mortality was observed at the site without a drawdown (Hall and Cuthbert, 2000). The cause of death of the turtles was determined to be primarily predation, as many carcasses exhibited gouges in the neck region, and death was



secondarily due to road mortality and winterkill (Hall and Cuthbert, 2000). In addition to increased mortality, the Blanding's turtles at the wetland drawdown site were also more mobile than those at the unaltered site, which has serious implications in areas where roads are present in close proximity to the altered wetland (Hall and Cuthbert, 2000). These findings are consistent to those of Anthonysamy et al. (2013), in which movement patterns of Blanding's turtles in Illinois were significantly altered between drought and non-drought years, increasing the risk of road mortality and vulnerability to other threats. It is clear that altering wetland properties has the potential to cause increases in mortality by multiple means.

#### *Implications of MMEs in an Increasingly Stochastic Environment*

In a time of less predictable environmental conditions as a result of rapid climate change, the ability of species to adapt to these changes is ultimately pivotal to their survival, and has become a prominent theme in evolutionary ecology (Botero, 2015). As is evident in my study and others, even short-lasting alterations can have drastic effects on survivability of otherwise robust individuals (Anthonysamy et al., 2013; Hall and Cuthbert, 2000; Ultsch, 1989). In the case of long-lived species such as chelonians, where the loss of only 10% of the adults in a population can wreak havoc for decades, the risk of local extinction is extremely high, and these organisms will be unable to sustain increasingly frequent catastrophes in the future (Botero, 2015; Brooks et al, 1991). As such, climate prediction models and associated alteration of environmental factors such as water levels of significant aquatic systems, should be considered when determining the risk of extinction for long-lived vertebrates. Where management action is to be taken, an understanding of the most efficient conservation strategies for long-lived vertebrates is of utmost importance, to minimize the potential for localized extinction, and to maximize allocation of

limited funding. Chapter 2 of this thesis investigates the potential effects that this MME may have on the persistence of the MBPP population, and assesses the effectiveness of several conservation initiatives.

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## Tables, Figures, Plates, and Appendices

### Tables

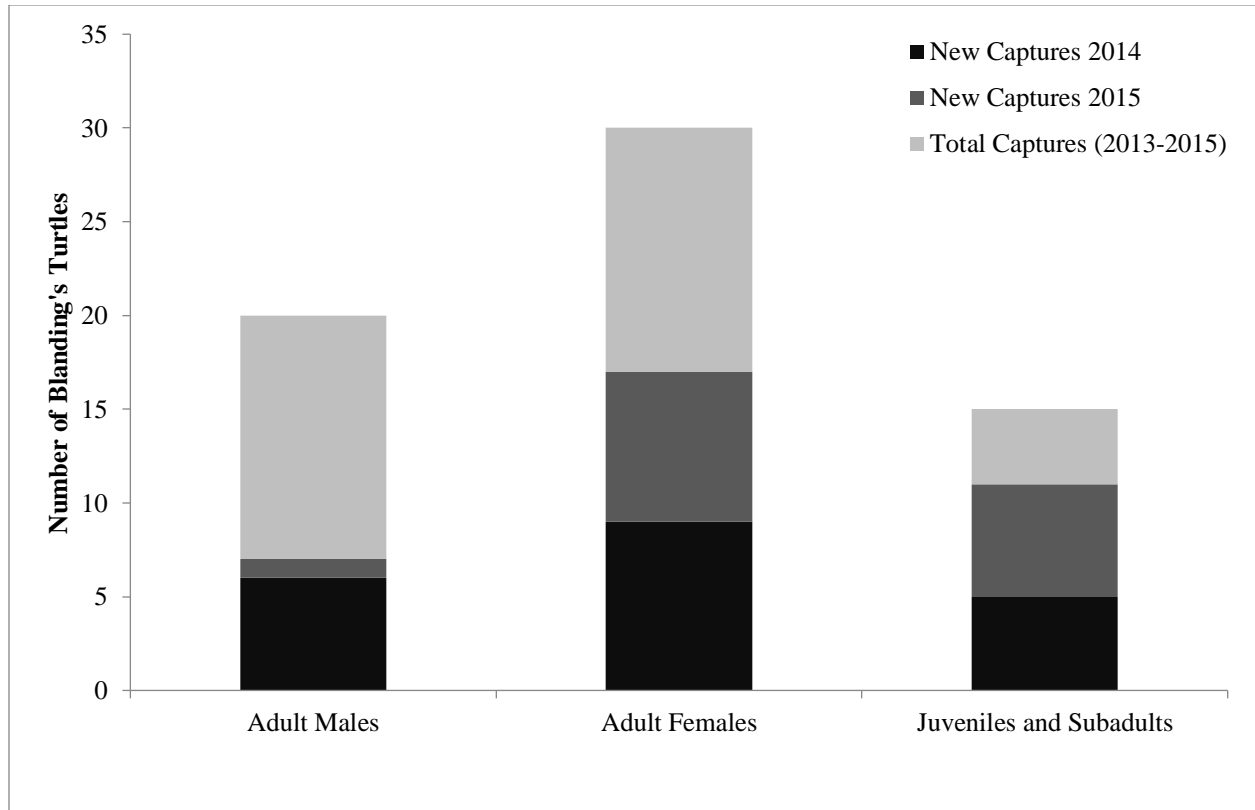
**Table 1.1:** Total number of the three turtle species encountered at Misery Bay Provincial Park from May-August 2014 and 2015, Blanding's turtles (*Emydoidea blandingii*), painted turtles (*Chrysemys picta marginata*), and snapping turtles (*Chelydra serpentina*), both dead and alive.

	Blanding's turtles	Painted turtles	Snapping turtles
Alive	47	16	4
Dead	53	13	3
<b>Total</b>	<b>100</b>	<b>29</b>	<b>7</b>

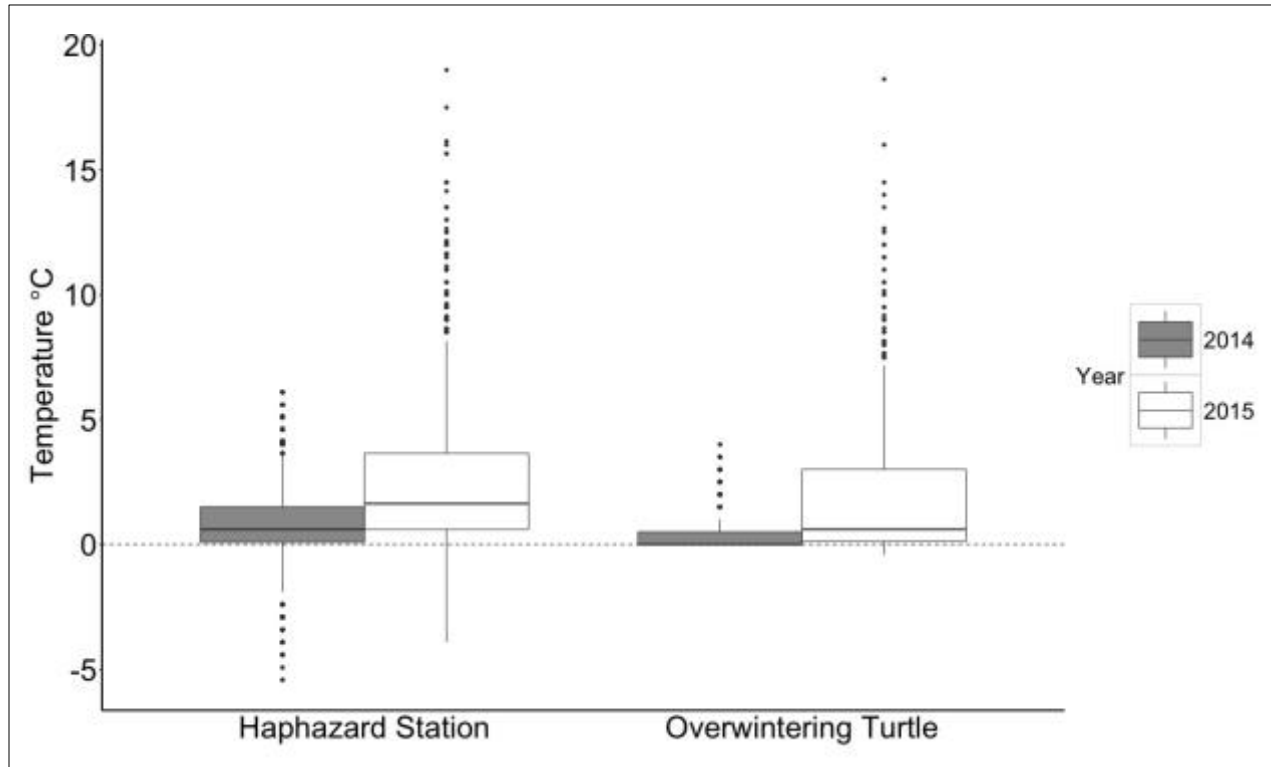
**Table 1.2.** Mean (SE) dissolved oxygen (mg/L) content at Misery Bay Provincial Park, in locations where Blanding’s turtles (*Emydoidea blandingii*) are known to overwinter (N = 6), where mortality occurred (N = 9), where no mortality occurred (N = 11), in the main fen area (N = 17), and in the southwest corner area (N = 3) during January, February, and March of the 2016 winter season.

	January	February	March
Overwintering Sites	13.87 ± 0.59	13.16 ± 0.82	13.94 ± 0.73
Mortality Sites	14.42 ± 0.38	13.62 ± 0.24	13.02 ± 0.76
Non-Mortality Sites	14.01 ± 0.40	13.21 ± 0.46	13.56 ± 0.45
Main Fen	13.95 ± 0.35	13.25 ± 0.33	13.34 ± 0.50
SW Corner	15.04 ± 0.59	14.21 ± 0.09	13.15 ± 0.13

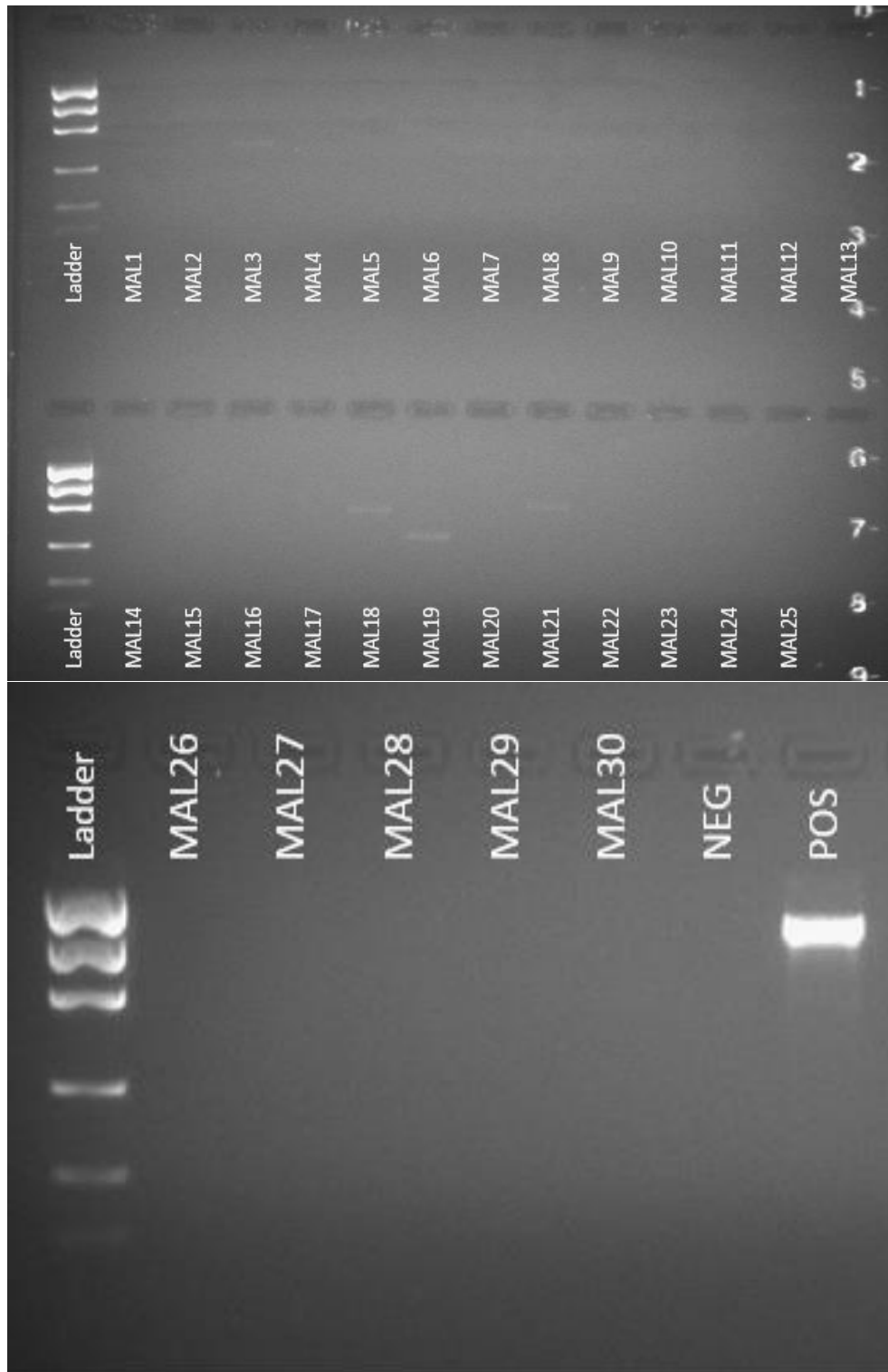
## Figures



**Figure 1.1:** Number of live Blanding's turtle (*Emydoidea blandingii*) captures in the 2014 and 2015 field seasons (May-September) in Misery Bay Provincial Park, Manitoulin Island, categorized by sex (male/female) and life history stage (adult/juvenile).



**Figure 1.2:** Boxplots of temperature recordings, occurring every 3 hours from 1 December to 31 March for both the 2014/15 and the 2015/16 winter seasons, of the haphazard stations (N = 31) and overwintering Blanding’s turtles (*Emydoidea blandingii*; N = 23) in Misery Bay Provincial Park, Ontario, Canada. The horizontal line within each box indicates the median, boundaries of the box indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, the whiskers indicate the high and low values, and the dots indicate outliers. The horizontal dotted line indicates 0°C. The lowest recorded temperature at haphazard stations was -5.4°C on 27 February 2015. No temperatures below 0°C were recorded by dataloggers on overwintering turtles. There was a significant difference in temperature between the overwintering turtles and the haphazard stations ( $t_{35} = -2.12$ ,  $p < 0.05$ ).



**Figure 1.3:** Images of resulting PCR gels, showing ladders, known negative (NEG) and positive (POS) samples, and 30 Leopard frog (*Lithobates pipiens*) samples (MAL1-30) collected from Misery Bay Provincial Park, Ontario.

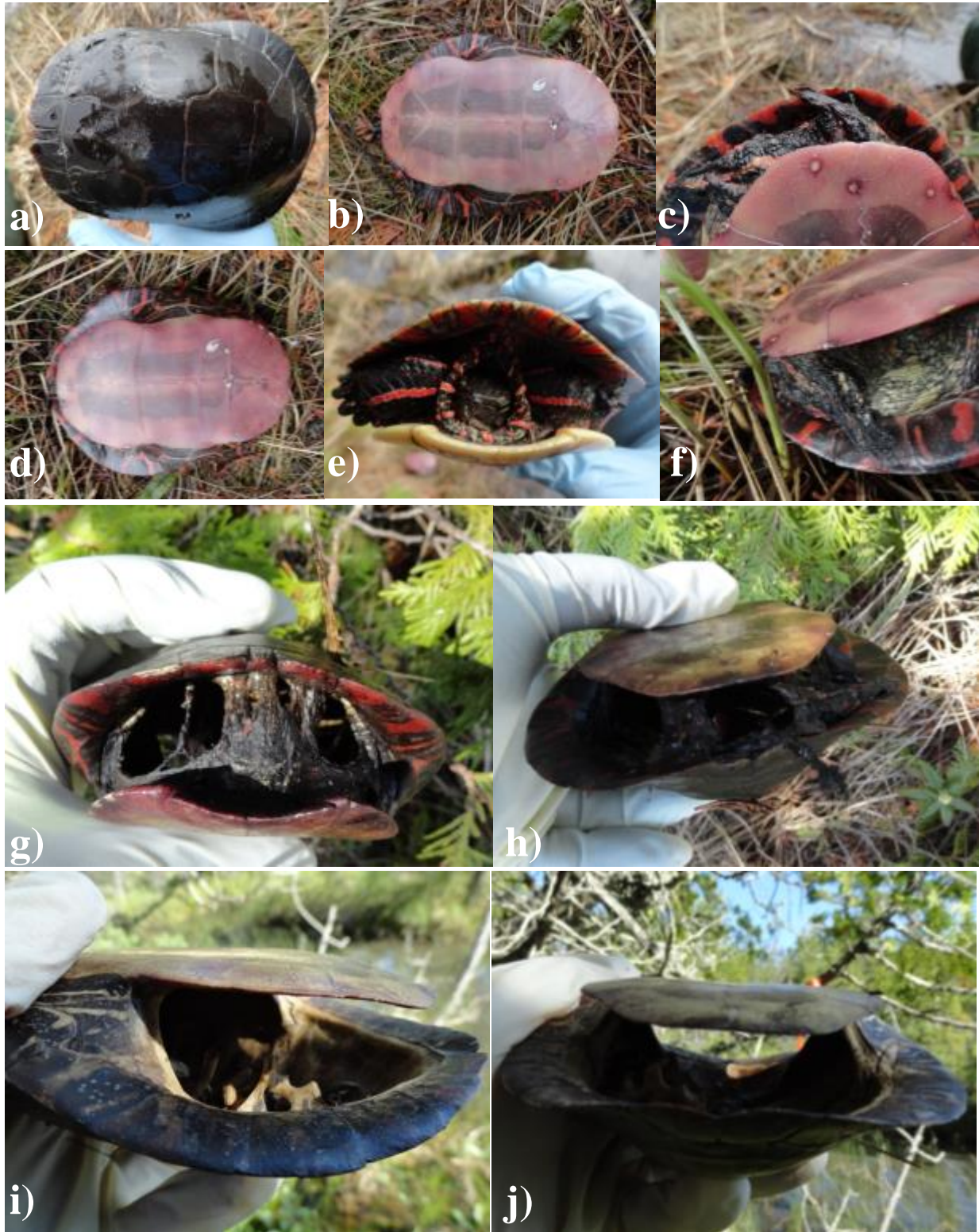
## Plates



**Plate 1.1:** Photographs taken of a dead Blanding's turtle (*Emydoidea blandingii*) as found on 18 July 2012 by Linda Reid (Misery Bay Provincial Park visitor). The front limbs and tail were intact, all in good condition, indicating that the carcass was found soon after death. The head was absent, and was described as being “gnawed off”. While not visible, the hind limbs were also attached to the body and in good condition.

- a) View of the turtle as it was found, plastron facing up, with forelimbs and tail visible.
- b) View of the carapace of the dead Blanding's turtle after the carcass was turned over.







**Plate 1.2:** A dead painted turtle (*Chrysemys picta*) found in the large fen associated with Misery Bay proper. The turtle was found at the edge of a small pond near the northwest border of the wetland, but outside of the park boundary. This turtle was found carapace-up and appeared to be in good condition, with the exception of missing toes and some shriveling at the distal ends of the missing limbs and tail. The right forelimb was present in full.

***11 May 2014***

- a) The carapace of the dead painted turtle. Scute sutures and keratin layer were firmly attached.
- b) The plastron of the dead turtle, with some teeth marks present.
- c) A posterior section of the plastron, showing markings present and initial decay at the distal ends of the tail and the right hind limb.

***22 May 2014***

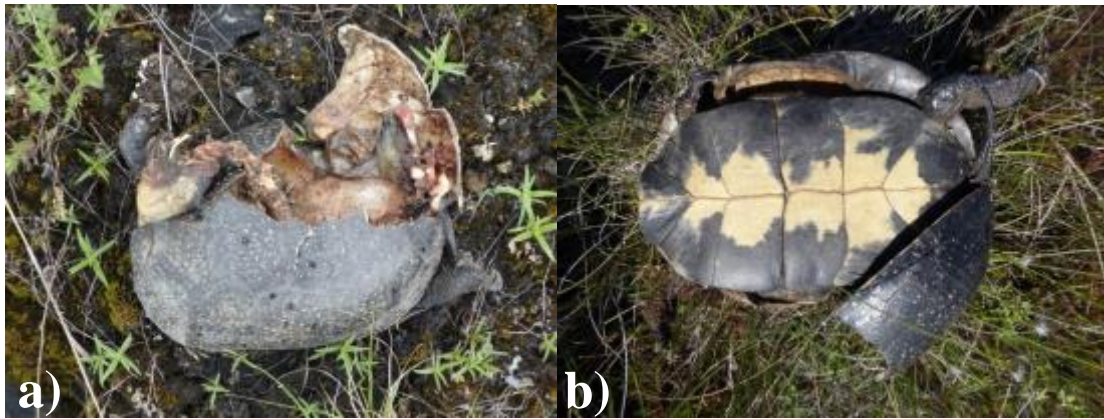
- a) The plastron of the dead turtle, showing slight colour change, specifically surrounding posterior marks.
- b) Anterior view. Head and right forelimb still in good condition. Shriveling evident at distal end of left forelimb.
- c) Left posterior hindlimb, in similar stage of decay of the right hindlimb (c) than on 11 May 2014.

***4 June 2014***

- a) Anterior view. Decay has progressed such that an empty space exists between the carapace and plastron of the shell.
- b) Posterior view. Note the change in plastron colour, whereby the outer edge has retained a pink colour while the center area has not.

***26 June 2014***

- a) Right posterior view. No soft tissue remained at this point. Also note the small bones present between the carapace and plastron, and the further loss of colour of the exterior shell surfaces.
- b) Anterior view. No soft tissue remained. Small bones contained between the carapace and plastron.



**Plate 1.3:** A Blanding's turtle (*Emydoidea blandingii*) that was found dead on a road outside of Misery Bay Provincial Park (MBPP), Ontario, presumably after being hit by a vehicle on 24 June 2016. The carapace had been broken open, and the internal organs and other materials were no longer present. The turtle was moved into the wetland to act as a decoy to attract potential predators so that they could be photographed by an associated motion-activated wildlife camera.

**26 June 2015**

- a) The dead Blanding's turtle near the site where it was originally found, with a cracked carapace and internal organs missing.
- b) The dead Blanding's turtle was positioned plastron-up in the main fen wetland of MBPP, to simulate the situation of the carcasses that were found in 2013.
- c) The carcass was paired with a nearby motion-activated wildlife camera, to capture photographs of any animals that showed interest in the carcass.

**28 August 2015**

- d) View of the carapace. The soft tissues have become desiccated, but are still present. The shell has maintained its integrity.
- e) View of the plastron. Note that the bone of the left hindlimb is void of flesh after being positioned such that it was submerged in water, while the skin and other soft tissues of the right forelimb, which was positioned out of water, are present and appear to have experienced only minor decay.

**1 May 2016**

- f) The shell of the Blanding's turtle had begun to disarticulate. The piece on the left side of the photograph was recovered from the water, while the piece on the right was out of water. Note the algal growth on the piece from the water, while the piece found on land is devoid of such growth.
- g) The pieces of carapace that were in the water had very loose association with the keratin layer of the scutes. A pink jelly layer remained between the keratin and bony elements of the shell.







STEALTH CAM 018F 12-31-2014 12:46:07  
 2015-07-21 8:33:18 AM M 1/3 21°C

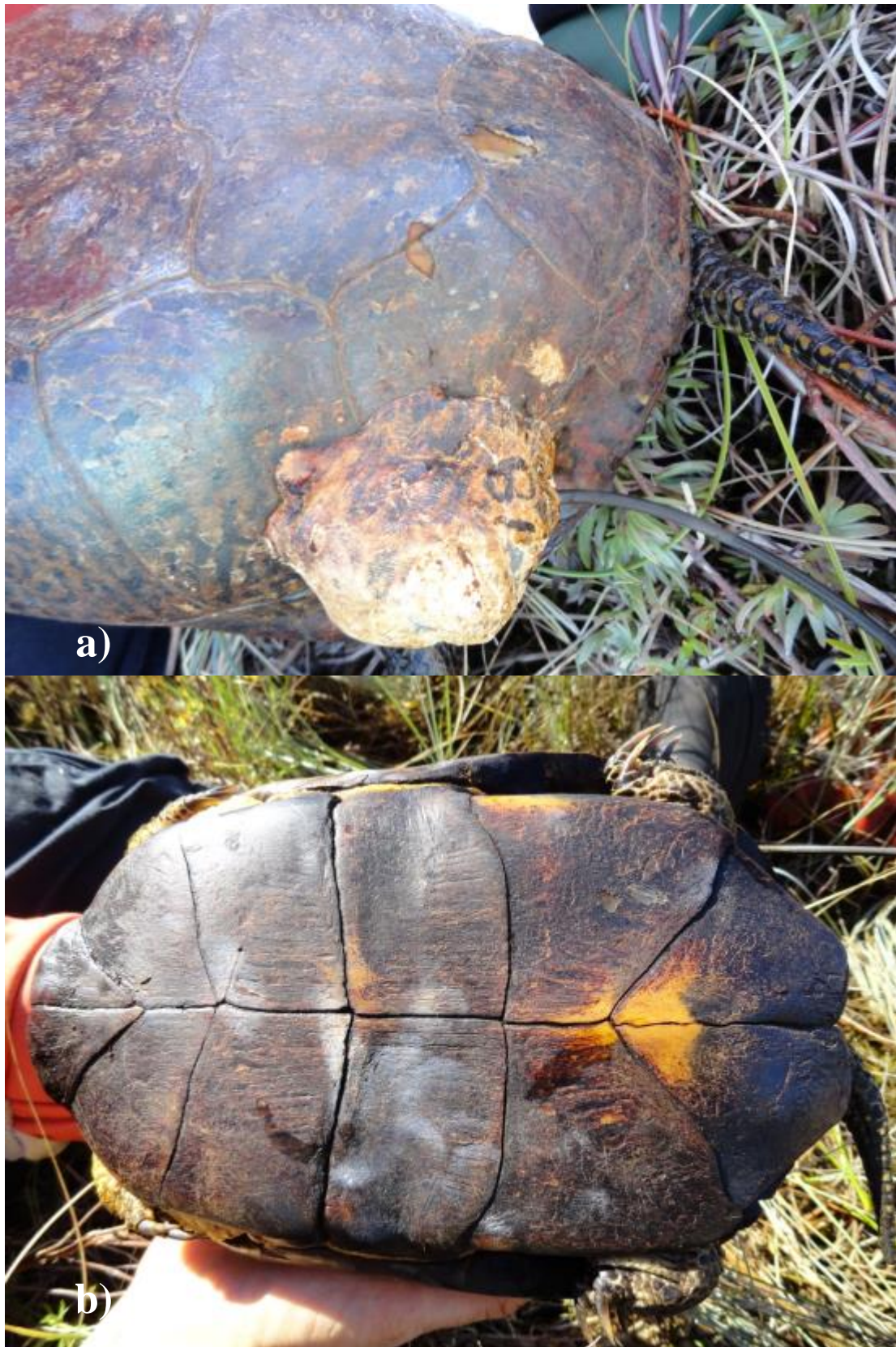


PC900 PROFESSIONAL RECONIX

**Plate 1.4:** Potential predators of Blanding's turtle in Misery Bay Provincial Park (MBPP), Ontario, as identified via photographs taken by motion-activated wildlife cameras.

- a) Three raccoons (*Procyon lotor*), each indicated by a red arrow, in the main wetland of MBPP.
- b) Raven (*Corvus corax*), indicated by the red arrow, in a SW corner wetland within MBPP.
- c) Coyote (*Canis latrans*) in the main fen wetland of MBPP.
- d) Four river otters (*Lontra canadensis*) in the SW corner area of MBPP.





**Plate 1.5:** Photographs of a live Blanding's turtle (*Emydoidea blandingii*) as found on 24 September 2014. The turtle showed evidence of a predation attempt around its transmitter, evident by the loss of some of the epoxy that covered the transmitter, and the damage to the keratin layer of the posterior area of both her carapace and plastron.

a) Dorsal view of the posterior of the carapace, showing damage to the keratin layer of the shell on the 5<sup>th</sup> vertebral scute and at the seam of the 4<sup>th</sup> vertebral and 4<sup>th</sup> costal scutes. Also visible is the grey covering of the transmitter, which had been uncovered from beneath a white layer of epoxy.

b) Ventral view of the plastron, showing damage to the keratin layer on the left femoral scute of the turtle.



## Appendices

### Appendix I

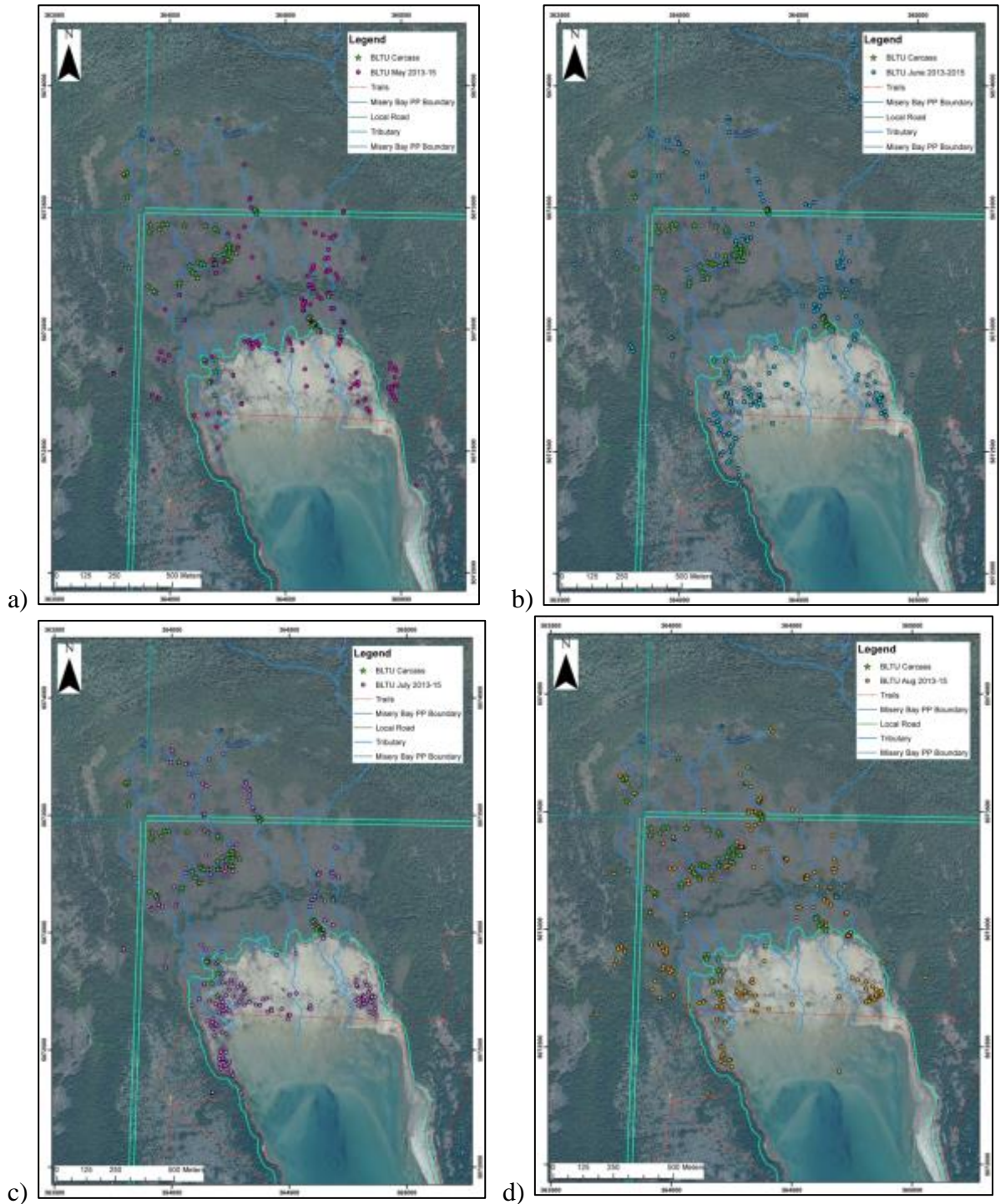
**Appendix I.I:** Initial and final concentrations of reagents in the master solution for Misery Bay Provincial Park, Ontario, Leopard frog (*Lithobates pipiens*) toe clip samples (N = 30), in 15  $\mu\text{L}$  reaction volumes. Excess of the master solution included for controls and to account for pipetting error.

Reagent	Initial	Final	Volume/Reaction ( $\mu\text{L}$ )	Volume for Master Solution ( $\mu\text{L}$ )
Buffer	5 X	1.00 X	3	126
dNTP's	10 mM	0.20 mM	0.3	12.6
MgCl	25 mM	1.50 mM	0.9	37.8
BSA	3 mg/mL	0.15 mg/mL	0.75	31.5
Forward	10 uM	0.30 uM	0.45	18.9
Reverse	10 uM	0.30 uM	0.45	18.9
Taq	5 U	1.00 U	0.2	8.4
DNA	-	-	4	-
ddH <sub>2</sub> O	-	-	4.95	207.9
<b>Total Volume</b>	-	-	<b>15</b>	<b>630</b>

**Appendix I.II:** Summary of species known to prey on turtles captured in photographs by motion-activated wildlife cameras stationed at multiple sites in Misery Bay Provincial Park, Ontario. Each animal was considered a new/unique individual if more than approximately 30 seconds elapsed between sequences of photographs of the same species. The activity and/or direction of travel of the animals was also taken into consideration when deciding whether multiple sequences had captured photographs of the same individual, or if they were multiple different individuals of the same species.

Location Description	Location (UTMs)	Date Positioned	Estimated Number of Individuals of each Species					Notes
			Coyote ( <i>Canis latrans</i> )	North American River Otter ( <i>Lontra canadensis</i> )	Raccoon ( <i>Procyon lotor</i> )	Raven ( <i>Corvux corax</i> ) or American Crow ( <i>Corvus brachyrhynchos</i> )	Non-Predatory Species (number of species)	
Hidden Forest Fen	363772, 5071431	January 26-March 8, 2016	-	-	-	11	1	
Hidden Forest Fen	363772, 5071431	August 11, 2015-March 5, 2016	-	-	-	-	1	
SW Beach Near Ditch	363773, 5071240	June 8-August 4, 2016	-	11	26	89	4	Positioned after seeing otter tracks at site
East Forest Fen	364973, 5072792	January 25-May 28, 2015	3	-	-	-	5	
Back Fen Hib Spot	364386, 5073472	November 13, 2014- April 16, 2016	2	-	5	-	2	
Zone of Death 2014 Previous Decoy Location	363966, 5071230	January 25-June 26, 2015	-	-	-	2	3	
Zone of Death #2	0364081, 5073295	August 3, 2015-March 5, 2016	-	-	-	-	2	
Zone of Death #3	0364020, 5073369	-	-	-	-	-	3	
Mink Trail Cam*	364232, 5073482	January 25-July 23, 2015	1	-	-	-	3	Decoy added May 24, 2015

Back Fen Decoy Cam*	036148, 5073491	July 23-Sept 30, 2015	-	-	1	-	4	Raccoon appears to be sitting on decoy
West Trib/Trail	364066, 5073035	January 26- June 24, 2015	3	-	6	-	4	
Staged Dead BLTU	364266, 5073309	June 24-Dec 8, 2015	5	-	-	-	4	Coyote removed Blanding's turtle carcass
Dead PNTU	363870, 5073808	May 11- August 19, 2014	-	-	1	1	4	In "Todd's Pond" (not within MBPP boundaries)
2014 Decoy1* West fen	363966, 5071230	July 4-Oct 22, 2014	-	-	-	1	3	
2014 Decoy2* SW Corner	036976, 5071235	July 4-Oct 22, 2014	-	-	-	-	2	
<b>TOTAL</b>	-	-	<b>14</b>	<b>11</b>	<b>39</b>	<b>104</b>	<b>45</b>	-



**Appendix I.III:** The location of Blanding's turtle carcasses found in 2013 (green stars) relative to the positions of live Blanding's turtles (*Emydoidea blandingii*) located via radio telemetry in 2013, 2014, and 2015, during the months of **a)** May (pink circles), **b)** June (blue circles), **c)** July (purple circles), and **d)** August (orange circles) in Misery Bay Provincial Park, Ontario, Canada.

## **Chapter 2:**

**Examining Long Term Consequences of a Mass Mortality Event in the Long-Lived Species,**

***Emydoidea blandingii***

## Abstract

Little information pertaining to long-term effects of mass mortality events (MMEs) exists in the primary literature, especially in relation to long-lived species such as chelonians. A MME of Blanding's turtles (*Emydoidea blandingii*) in Misery Bay Provincial Park (MBPP), Ontario, Canada, was first reported and has been under investigation since 2013. Being aware of the causes of each MME aids in planning successful recovery strategies. With 53 dead ( $N_{\text{adult}}=44$ ,  $N_{\text{subadult}}=6$ ,  $N_{\text{juvenile}}=2$ ,  $N_{\text{unknown}}=1$ ) and a current population estimate of 47 live resident Blanding's turtles, this event appears to have removed over half of the resident breeding population. Adult survivorship is essential for population persistence, as age at first reproduction is at least 14 years for males and 18-22 years for females, and vulnerable eggs and juveniles experience high mortality rates in this species. Given the life history of Blanding's turtles, it is expected that the population at MBPP will continue to decline if unaided. Population viability analyses were conducted to determine the most efficient recovery strategy, and found that nest protection, the introduction of juveniles, and the introduction of adults were each increasingly successful, though overall none of these strategies resulted in stable or positive population growth far into the future. The most successful strategy tested was a combination of nest protection and annual introduction of 25 female turtles at two years of age over a 50-year period. The information gained through this study has led to the recommendation of potential conservation strategies for this population, and will aid in the management of future MMEs elsewhere.

## Introduction

Little information pertaining to long-term effects of mass mortality events (MMEs) exists in the primary literature, especially in relation to long-lived species such as chelonians. Beyond understanding the causes of MMEs, it is imperative that we can assess their long-term effects on populations of long-lived species in order to ensure that these populations persist into the future and that effective recovery measures, if any, can be taken to do so. MMEs in species with long life history strategies, such as turtles, are of particular concern as these animals lack density-dependent population responses to increased mortality (Brooks et al., 1991; Keevil, unpubl. data).

### *Assessing the Likelihood of Population Survival after a MME*

Accurately assessing population viability to make inferences at the species level has become an important management practice in conservation biology (Akçakaya and Sjogren-Gulve, 2000). Tools such as population viability analyses (PVAs) have been honed in recent years, allowing us to better comprehend the long-term effects of MMEs. These tools provide an insight into the future of a population that can then be used by policy-makers. Unfortunately, long-term data required to make accurate population projections are rare for turtle species, limiting predictions about population longevity (Famelli et al., 2012; Enneson and Litzgus, 2009). However, some life history traits are likely highly conserved, remaining consistent throughout a species' range, and can be applied when population-specific life history data are lacking. In addition to knowledge of a species' life history, an accurate account of a catastrophe, such as a MME, is necessary in formulating accurate predictions (Coulson et al., 2001).

Moreover, using information gained from elsewhere in a species' range allows the projections of a PVA to be applied on a wider scale, rather than a fine-tuned projection of one specific population. For this reason, it is useful to incorporate data from other locations when population-specific data are lacking. While this may not provide the most accurate scenario for the population under scrutiny, it will provide results that are applicable to more populations. It is imperative that population projections and likelihood of survival analyses be used in assessing the conservation status of populations of at-risk species that have faced a MME. Considering that MMEs are rare events, and that ecological studies are generally quite short as compared to the lifespan of these species, the chance of witnessing such an event is highly unlikely (Fey et al., 2015; Reed et al., 2003). However, there has been an increase in reports of MMEs in recent years, coinciding with the decline of reptile species worldwide (Fey et al., 2015; Böhm et al., 2013). Freshwater turtle declines are particularly evident in Ontario, as the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) classifies seven of the province's eight native turtle species as at some level of risk of extinction (COSEWIC: [http://www.cosewic.gc.ca/eng/sct5/index\\_e.cfm](http://www.cosewic.gc.ca/eng/sct5/index_e.cfm)).

A MME of Blanding's turtles in Misery Bay Provincial Park (MBPP) on Manitoulin Island, Ontario was first reported in 2013, and has been under investigation since that time (see Chapter 1). This MME appears to have removed roughly half of the breeding population, as 53 ( $N_{\text{adult}}=44$ ,  $N_{\text{subadult}}=6$ ,  $N_{\text{juvenile}}=2$ ,  $N_{\text{unknown}}=1$ ) carcasses and 63 live ( $N_{\text{adult}}=46$ ,  $N_{\text{subadult}}=5$ ,  $N_{\text{juvenile}}=12$ ) Blanding's turtles, including 47 residents (defined as those that overwinter in MBPP), and 16 transients passing through the main fen area of the park, were found. Inspection of the carcasses



has shown that there were 22 female, 10 male, and 21 carcasses of unknown sex, due to the extent of decomposition that had occurred before the remains were found.

Little progress has been made in quantifying the severity of MMEs in natural environments (Reed et al., 2003), and remarkably few examples of PVAs incorporating MMEs exist (Mangel and Tier, 1994). Thus, the data collected after the MME at MBPP are exceptional and will allow us to gain a better appreciation of the role that these events play in a population's persistence. When considering the long-term consequences of this MME, it is possible that in the absence of human intervention, the MBPP Blanding's turtle population will be unable to persist, as such a drastic decrease in the number of egg-laying females per year will result in insufficient recruitment of young turtles needed to replace existing adults (Congdon et al., 1993; Brooks et al., 1991). While the immediate effect of this MME is obvious, the long term consequences are less clear. For example, this previously large population (as compared to Keevil, unpubl. data and COSEWIC, 2005) may have been able to tolerate a low number of females laying eggs each year, but with no density-dependent compensation (Brooks et al., 1991) it will not be able to increase reproductive output to maintain adequate recruitment of young into the population. Ultimately, this may result in the decline and eventual extirpation of the Blanding's turtle population in MBPP.

### *Objectives, Hypotheses, and Predictions*

The objective in my study was to examine the long-term effects of MMEs on long-lived chelonian species through the use of PVAs and the MBPP population of Blanding's turtles as an example. Life-history data were supplemented from other populations where necessary, as no

previous studies had been conducted on the MBPP Blanding's turtle population. Given the life history of Blanding's turtles (Congdon et al. 1993), I hypothesized that the main fen population of Blanding's turtles at MBPP will continue to decrease if left unaided. If this hypothesis is correct, the population may become extirpated unless recovery action is taken through the implementation of conservation strategies. Thus, a second objective of my study was to model effects of conservation strategies, including nest protection, juvenile supplementation, and adult supplementation, on post-MME population growth, to determine the most effective course of action to ensure population persistence.

## **Methods**

### ***Study Site***

MBPP is situated on the southern shore of Manitoulin Island, which contains a globally rare terrestrial ecosystem type called alvar, which is mainly found in the Great Lakes region of Canada and in some Scandinavian countries (Morton and Venn, 2000). An alvar is an environment formed on flat limestone bedrock, thin substrate is typical where substrate is present, supporting an assemblage of rare prairie/grassland flora and fauna (Morton and Venn, 2000). This study focused on two main areas within MBPP (Figure 0.2). The "main fen" area is north of Misery Bay proper and includes three large wetlands (~90 ha in total), two of which (a patterned fen and a marsh-fen) are separated by a historic beach ridge which runs east-west and is identified by the surplus of substrate and large trees growing along it, effectively dividing the wetlands. The hydrological properties of the coastal marsh are directly dependent on water levels of Lake Huron, while water levels in the marsh and patterned fens are dependent on precipitation and groundwater within the watershed, which is indirectly influenced by the water levels of Lake Huron. South of the marsh-fen is a coastal marsh, separated from the marsh-fen by a low beach

ridge. The north fen areas (~70 ha) are saturated with water, and reach maximum depths of approximately 75 cm in several pools throughout the area north of the beach ridge, as well as in the seven tributaries that run in a north-south direction through both fens. These tributaries flow into a coastal marsh area that transitions into open bay waters. Park trails avoid these wetland areas, as they are difficult to access without proper equipment and are very sensitive to damage done by excessive trampling. The turtle carcasses were largely found in this north fen area, primarily associated with the tributaries.

A secondary area in which Blanding's turtles have been found, termed the "southwest (SW) corner" includes several small wetlands/vernal ponds to the west of Misery Bay proper (Figure 0.2). No carcasses associated with the MME were found in this area of the park. Of these wetlands, one is a fen, and the others are largely situated on depressions of alvar rock where water accumulates in the spring and early summer. Some of these routinely dry up as the summer season progresses, depending on weather conditions, but a few are directly supplied by overflow from Lake Huron, dependent upon precipitation and Lake Huron water levels, and contained at least 60 cm of water throughout the summer season in 2014 and 2015. Park trails avoid two of these wetlands, which are concealed within the forest, but trails do come in close proximity of one vernal pond and two coastal pools that are used by the turtles in the summer months (Appendix I.III).

### ***Population Viability Analysis***

The population viability analysis (PVA) software Vortex, version 10.0.7.9 (Lacy and Pollak, 2014) was used to conduct the PVA for this study. Vortex allows for the modeling of population

dynamics that take into account random variables and species-specific parameters that describe an annual cycle of the chosen organism (Lacy et al., 2015; Lacy 1993). Simulations are iterated many times to illustrate a distribution of fates that the population may endure (Lacy et al., 2015). Since some migration and mating occurs between individuals of the main fen and SW corner areas, I modeled the two MBPP demes in the PVAs. However, recovery actions were only applied to the main fen area, as no mortality occurred in the SW corner area of the park. Furthermore, the results and associated figures focus primarily on the viability of the main fen deme.

### ***Input Parameters***

#### *Population Size Estimate and Initial Population Size*

A population size estimate for the SW corner area of MBPP was generated using a simple count system, as the area is small, has good visibility, and no new turtles have been found in this area since initial capture and marking efforts in 2013, indicating that, while a small number of migrants are likely to pass through during the active season, all resident turtles have been found and marked.

A current (post-MME) population size for the main fen area of MBPP was estimated using data collected during the mark-recapture and haphazard surveys for Blanding's turtles during the month of May in the 2013, 2014, and 2015 field seasons (three sampling occasions). The month of May was selected to exclude transient turtles, enabling the analyses to focus solely on resident turtles. If turtles who disperse into MBPP from other demes later in the summer were included, they would deceptively inflate the resulting estimate. Transients could not be accurately

incorporated into the analyses, as population dynamics of demes outside of the park were not obtained through this study. A pre-MME population size estimate was generated by adding the number of Blanding's turtle carcasses found to the current population size estimate of the main fen area of MBPP. This was done because the capture probability of the carcasses is unknown, but is assumed to have been higher than that of live turtles. The study site size was consistent between each of these years, which is a crucial assumption (Cooch and White, 2015).

A Cormack-Jolly-Seber model, based on the POPAN option in Program MARK (White and Burnham, 1999), was used to generate the population size estimate. The POPAN model integrates survival ( $\phi$ ), capture probability ( $p$ ), probability of entry ( $p_{ent}$ ), and population size ( $N$ ) in generating a population size estimate (Cooch and White, 2015). To incorporate individuals who were part of the telemetry study (and whose capture probability ( $p$ ) was equal to 1.00), four groups were created: Group 1) Turtles found without transmitters in 2013, transmitter present in 2014 and 2015, Group 2) Turtles found without transmitters in 2013, transmitter present in 2014, but not in 2015, Group 3) Transmitter present in 2015 only, and Group 4) Found without transmitters in 2013, 2014, and/or 2015, transmitter never present. Three POPAN models were fit to the data, each of which had time-dependent probability of capture to incorporate the aforementioned groups. Models differ in combinations of fully time-dependent and constant (except for probability of capture) model parameters (survival, probability of entry, and population size). These models were compared using Quasi-Akaike's Information Criterion (Cooch and White, 2015; Table 2.1) A post-MME population size estimate of 47 individuals was incorporated into the PVAs except for the "No-MME" scenario, which describes the population

as if no MME had ever occurred, and which had an initial population size of 100 individuals ( $N_{\text{dead}} = 53 + N_{\text{alive}} = 47$ ).

### *Proportion of Breeding Adults*

The proportion of breeding adults was estimated separately for each sex. Since the investment into mating and siring offspring is significantly less for males, 100% of the males were estimated to be participating in breeding in any given year. Female investment in breeding is higher than that of males, and the proportion of breeding females in any given year was estimated based on observations during the 2014 and 2015 summer field seasons, in which only four of the resident radio-tagged females laid nests over the study period, with only one of these four individuals nesting in both the 2014 and 2015 field seasons. As such, the proportion of breeding females was set to 25% (1/4 females in the telemetry study nested in both 2014 and 2015) for the main fen and 37.5% for the SW corner area (1/2 and 1/4 females in the telemetry study nested in 2014 and 2015, respectively) of MBPP (Table 2.2). While at the extreme low end, this proportion is within that of Blanding's turtle populations reported elsewhere (23-85%; Ernst et al., 2009; Congdon et al., 1993; Congdon et al., 1983). Reproduction was set to be density-independent.

### *Age of First Reproduction, Both Sexes*

While sexual maturity is typically attained once a Blanding's turtle has reached a minimum straight-line carapace length of 152 mm, which corresponds with an age at maturity of at least 14 years, the attainment of sexual maturity in Blanding's turtles may be delayed to up to 25 years in the northern regions of their range (COSEWIC 2005; Bury and Germano 2003; Congdon et al. 2001). As such, sexual maturity of Blanding's turtles was estimated to occur at 20 years of age

for my PVAs. Blanding's turtles are not known to experience senescence, and will continue to breed throughout their lifetime (Congdon and van Loben Sels, 1993). The oldest known Blanding's turtle is 83 years old (Science Daily, 2016), and this was considered the maximum age at reproduction, and the maximum lifespan.

### *Annual Harvest*

The MBPP Blanding's turtle population is situated in a protected park, which is void of many anthropogenic causes of death that Blanding's turtle populations face elsewhere in Ontario, such as road mortality. However, there is evidence that low levels of human-caused mortality does occur, namely when turtles venture outside of MBPP boundaries. Only a few turtles were observed to leave the boundaries of the park each year during the three-year radio telemetry study. Small amounts of mortality occur from collision with vehicles as some turtles cross roads surrounding MBPP. Females who were part of the telemetry study crossed private cottage access roads, and none were struck; however, two females who were not part of my study (no evidence of notching) were hit and killed by vehicles on these low-use roads. Additionally, one female was recorded crossing Misery Bay Park Road, the road that visitors must use to access the park Visitor Center and the hiking trails. Males who left the park crossed either Misery Bay Park Road or the two-lane highway (HWY 540) north of the park. No males outfitted with transmitters succumbed to road mortality during the study. This evidence suggests that road mortality occurs on a small scale at this site. As such, harvest was incorporated into each PVA to determine the impact that small, recurring removal of adults, including breeding females, has on the population. The harvest for each scenario was the same, but differed between the two areas of MBPP to represent the different risk of mortality. The main fen was modeled with a harvest of 1

female and 1 male every 3 years and the SW corner was modeled with a harvest of 1 female and 1 male every 5 years.

#### *Average Clutch Size*

Clutch size is known to vary in Blanding's turtles, whereby larger females tend to have larger clutches and larger eggs within their clutch (Rowe, 1994; Congdon and van Loben Sels, 1993; Gibbs, 1982;). Average clutch size in Michigan was 10.2 eggs/clutch (Congdon et al. 1983), and maximum reported clutch size is 15 eggs/clutch (Standing et al., 2000). While some nests were found on property surrounding MBPP, a confident estimate for average clutch size has not been obtained for my study population. Eggshells were counted outside of dug-up nests, but in some cases there were no eggshells remaining. Some nest predators, such as Corvids, have been known to eat the eggshells of painted turtles (*Chrysemys picta*), removing evidence of the number of eggs deposited (Rollinson and Brooks, 2007). I used the maximum clutch size of 15 eggs/clutch (Standing et al., 2000) in my PVAs.

#### *Breeding System and Sex Ratio at Hatch*

Blanding's turtles display a breeding system in which there are multiple paternities per clutch (Refsnider, 2009). Furthermore, observations in the field suggest that males in the MBPP Blanding's turtle population are polygynous, and so a polygynous breeding system was incorporated into my PVAs. The sex ratio of hatchlings was estimated to be 1:1.



### *Carrying Capacity*

The carrying capacity is the maximum population size that a particular ecological system can sustain indefinitely, by providing adequate food, water, habitat, and other required resources (Morris and Doak, 2002). The carrying capacity of MBPP is unknown, but density of Blanding's turtles for three ecoregions of Ontario (Lake Erie/Lake Ontario, Simcoe/Rideau, and Georgian Bay) were 0.78 adults/ha, 0.29 adults/ha, and 0.12 adults/ha, respectively (Keevil, unpubl. data). Since MBPP is a unique habitat system for Blanding's turtles (Sheppard, 2014), it is difficult to estimate a realistic carrying capacity. To minimize the effect of unknown biological factors, the carrying capacity of MBPP was set to 500 individuals (approximately 5.56/ha), which is far above the pre-MME population size estimate, so that the carrying capacity, which cannot be accurately estimated, does not interfere with the projections of the models. The PVA was set to project a population that is not density-dependent.

### *Survivorship at Various Life Stages*

Chelonians, including Blanding's turtles, are known to experience high mortality rates in their early life stages, starting with the egg stage (COSEWIC, 2005; Congdon and van Loben Sels, 1993; Congdon, et al., 1993). However, it is recognized that the risk of mortality decreases substantially with age and size (COSEWIC, 2005; Congdon, et al., 1993; Brooks, 1991). Thus, once Blanding's turtles reach their adult age and body size, they generally persist in the population for a very long time. The values for survivorship of Blanding's turtles at various life stages in life table presented by Congdon et al. (1993) were used (Table 2.2).

### *Quasi Extinction Threshold*

As was done in the study on spotted turtles (*Clemmys guttata*) conducted by Enneson and Litzgus (2009), population extinction was set to occur when the population reached a minimum size of 8 individuals. A quasi-extinction threshold was used, as opposed to an absolute extinction threshold of zero individuals, as factors such as genetic viability and ability to locate suitable mates increase the probability of extinction in populations existing at very low densities (Morris and Doak, 2002).

### ***Base and Additional Models***

All PVA models were iterated 500 times over various timespans in 25 year increments. The base model represents the current population at MBPP, which experienced a MME and has not had any type of conservation or recovery strategy applied (Table 2.3). The other models include one in which no MME occurred, and four where recovery actions occurred, including nest protection, introduction of juveniles into the main fen population, introduction of adults to the main fen population, and a combination of nest protection and introduction of juveniles into the main fen population (Table 2.3).

In the case of nest protection, the input parameter for mortality at age 0-1, which represents the period of time between egg laying and hatching, was decreased from 0.74 (Congdon et al., 1993) to 0.00. The scenario involving the introduction of juveniles simulates an introduction of 50 juvenile (two years of age; captive bred) female Blanding's turtles each year for 25 years, beginning in year 5 and ending in year 30, as has been proposed by the Toronto Zoo for their urban population of Blanding's turtles (Yannuzzi, pers. comm., 2016; Toronto Zoo, 2015). The

scenario detailing recovery action through the introduction of adults describe the addition of 25 adult females into the population every 10 years, beginning in year 20 and ending in year 70 (Table 2.3), to incorporate time taken for these turtles to reach maturity. The scenario involving the combination of nest protection and introduction of juveniles simulates an introduction of 25 female juvenile (two years of age) Blanding's turtles each year for 25 years, beginning in year 5 and ending in year 30.

### *Sensitivity Analyses*

I isolated hatchling mortality and juvenile and adult supplementation through a sensitivity analysis, to assess the relative importance of each parameter (Middleton and Chu, 2004). This enabled a fine-tuned perspective of the consequences that various recovery strategies will have on long-term persistence by estimating time to extinction. A base model included the MME, but no recovery strategies. Hatchling mortality and juvenile and adult supplementation were incorporated as parameters and tested using Latin Hypercube Sampling (LHS). LHS is more efficient than random sampling, as it more evenly covers the parameters (Lacy et al., 2015).

## **Results**

### *Population Size Estimate*

The highest ranking model included constant survival, with a Quasi-AIC weighting of 80.4 (Table 2.1). The post-MME population size estimate of the main fen area of MBPP was 47 individuals (35.85-64.29, SE = 7.12). The pre-MME population size estimate was simply the total number of Blanding's turtle carcasses found ( $N = 53$ ) added to the post-MME population estimate, resulting in an estimate of 100 individuals in the main fen area of MBPP. As previously

described, the population size estimate for the SW corner was generated using a simple count, and resulted in an estimate of 13 individuals in this area of MBPP.

### ***Population Viability Analyses***

#### ***Base Model***

The parameter values in Tables 2.2 and 2.3 define the base model and subsequent models, respectively. The probability of the main fen deme experiencing extinction in a 50-year projection of the base model was 100%, with a mean instantaneous growth rate ( $r$ ) for all years of -0.068 (SD = 0.139; Figure 2; Table 2.4). The SW corner deme was also projected to become extinct in a 50-year projection of the base model, following a similar pattern to that of the main fen deme (Figure 2).

#### ***No Mass Mortality Event***

The model describing the main fen deme as if it did not undergo the mass mortality event had a 0% probability of extinction in a 25-year projection. Under these conditions,  $r = -0.026$  (SD = 0.097; Table 2.4). However, this scenario projected a probability of extinction of the main fen population of 100%,  $r = -0.056$  (SD = 0.118) in 75 years, 25 years later than the base model projection. The SW corner deme was projected to follow a similar pattern as that described in the base model, whereby extinction is reached approximately 35 years into the future.

#### ***Nest Protection***

In the nest protection scenario, the probability of extinction of the main fen deme was 1% in a 25-year projection and  $r$  was -0.007 (SD = 0.196; Table 2.4). In a 50-year projection, the

probability of extinction rose to 71% ( $r = -0.035$ ,  $SD = 0.203$ ; Table 2.4), and probability of extinction reached 100% in a 100-year projection ( $r = -0.040$ ,  $SD = 0.205$ ; Table 2.4), 50 years later than that described by the base model projection. Once again, the PVA projected that the SW corner deme would experience extinction approximately 35 years into the future.

### *Introduction of Juveniles*

In the scenario in which 50 individual two-year-old juvenile females were added to the main fen deme each year for 25 of years, this deme had a probability of extinction of 0% after 50 years, with  $r = 0.067$  ( $SD = 0.144$ ; Table 2.4). The model was run for projection increments of 25 years, and after 100 years the main fen deme reached a probability of extinction of 100%, with  $r = -0.024$  ( $SD = 0.132$ ; Table 2.4). The SW corner deme was again projected to follow a similar pattern as that described in the base model, whereby extinction is reached approximately 35 years into the future.

### *Introduction of Adults*

In the scenario in which 25 adult (15 years of age) females were added to the main fen deme every 10 years for 50 years (Table 2.4), this deme experienced a 0% probability of extinction up to the 75-year projection ( $r = 0.001$ ,  $SD = 0.200$ ; Table 2.4). A 99% probability of extinction of the main fen deme was not reached until the 175-year projection, with  $r = -0.017$  ( $SD = 0.174$ ; Table 2.4), much later than the projection based on the base model. The SW corner deme was projected to follow a similar pattern as that described in the base model, whereby extinction is reached approximately 35 years into the future.

### *Nest Protection and Introduction of Juveniles*

The output from this scenario shows a 0% probability of extinction of the main fen deme in the 25 and 50 year projections ( $r = 0.050$  and  $0.011$ ,  $SD = 0.155$  and  $0.163$ , respectively; Table 2.4). The projections were run in 25 year intervals ending at 250 years into the future, at which point the probability of extinction of the main fen deme was 64% ( $r = -0.008$ ,  $SD = 0.177$ ; Table 2.4). The SW corner deme was projected to experience greater viability in this scenario, reaching extinction at approximately 45 years into the future under these conditions.

## **Discussion**

### *Post-Mass Mortality Event*

The post-MME, current population size of Blanding's turtles in MBPP was estimated to be 47 individuals in the main fen and 13 individuals in the SW corner. The pre-MME population size estimate was 100 individuals in the main fen, and 14 in the SW corner. One dead turtle was found in the SW corner area during 2015 field season, but the cause of death was determined to be unrelated to the MME. The SW corner area did not appear to experience any mortalities during the MME. The base model, which projected Blanding's turtle population size without the application of recovery strategies, showed that the probability of extinction was 100% in as few as 50 years, primarily as a result of insufficient recruitment to replace the aging adult population.

### *No Mass Mortality Event*

When the population was modeled as if there was no MME, the probability of extinction after 25 years was 0%, but reached 90% and 100% in the 50 and the 75 year projections, respectively, due to insufficient recruitment. An important consideration is that the extremely low

reproductive rates that I used, while within the limits of other Blanding's turtle populations (Ernst et al., 2009; Congdon et al., 1993; Congdon et al., 1983), may have been biased by the radio-tagged subset of the population that they were based upon. Increasing the proportion of females nesting annually in the model resulted in persistence of the population farther into the future (see Appendices II.I; II.IIa-f).

### *Nest Protection*

Nest protection is a popular conservation strategy employed for both freshwater and marine turtle species, usually involving the caging of nests with either above- or below-ground fencing, which excludes predators from individual nest sites. Riley and Litzgus (2013) found that nest caging does not affect the environmental nest conditions or alter proxies for hatchling fitness, and so nest cages are ultimately an effective conservation tool for preventing predation. Nest protection ensures that the embryo within the egg survives to hatch, barring any genetic irregularities/significant mutations, adverse environmental conditions, and/or parasitoid colonization. When the post-MME population was aided through the simulated protection and successful hatch of all nests, the population had a probability of extinction of 71% in only 50 years, and reached a 99% probability of extinction 75 years, similar to that of the unaided population. These model outcomes suggest that nest protection alone is not a viable recovery strategy for the MBPP Blanding's turtle population. In reality, all nests could not be protected due to the cryptic nature of nesting females, the low detectability of nests in a landscape, and the aforementioned threats to eggs that cannot be countered through the use of predator exclusion cages. Even if all nests were protected with predator exclusion cages, the model outputs indicate that the population decline would be minimally relieved, as is typical of turtle species with low

fecundity (Congdon et al., 1994). In addition, even if nests are protected from predators, there are still many threats to the hatchling and juvenile life stages of turtles, and mortality rates are estimated at 22% until age 14 (Freedberg et al., 2005; Heppell et al., 1996; Congdon et al., 1993). Thus nest protection should not be considered as a sole recovery action, as the population decline was not sufficiently stifled, even when all simulated nests were protected.

Furthermore, nest cages may not be practical at MBPP. Blanding's turtles in MBPP nest in built-up substrate in cracks and depressions on limestone alvar, making it difficult to secure cages without damaging the eggs. While cages can be weighed down from above by objects such as rocks, this design is easier to dismantle, by humans and predators, than those that have fencing present below and/or above the substrate surface. An additional challenge in nest protection at this site is that none of the gravid Blanding's turtles who were tracked for three years (N=5, including one female who was not a resident of MBPP, and one resident individual that nested in multiple years) were found to nest within the boundary of MBPP, meaning that special permissions would be necessary to implement nest-protection structures on private property. Nesting females typically travel far distances in search of adequate nest sites (Miller and Blouin-Demers, 2011; Congdon et al., 1983). While two main nesting areas have been identified and used by female residents of MBPP, it is possible that some resident females may nest elsewhere, and they would not likely be found through haphazard searches. Telemetry surveys of gravid females would overcome this challenge, but would require females being captured and equipped with transmitters prior to the nesting season. These nesting areas are relatively remote and would require personnel to remain on site throughout the Blanding's turtle nesting season, which can last up to one month, to conduct telemetry and/or haphazard surveys to locate cryptic egg-laying



females. For these reasons, and those described previously, the protection of nests using traditional designs is not recommended as the most effective recovery strategy for the MBPP Blanding's turtle population, mainly because it does not provide a sufficient boost for the population, and also because it would be difficult to implement and maintain the predator exclusion cages that have been used elsewhere.

### *Introduction of Juveniles*

The introduction of juveniles into a wild population typically occurs through head-starting programs, which involve the collection of eggs from wild nests, incubation until hatching, and captive rearing of hatchlings until their body size and strength is sufficient to lessen the impacts of natural threats (Heppell et al., 1996). The modeling scenarios that included supplementing the population with juveniles followed a similar protocol to that planned by the Toronto Zoo, whereby 50 juvenile Blanding's turtles were released at two years of age, over a period of 20 years (Yannuzzi, pers. comm., 2016; Toronto Zoo, 2015). Blanding's turtles exhibit temperature-dependent sex determination, whereby eggs incubated at or below 28°C will hatch as males, and eggs incubated above 29°C will result in females (Gutzke and Packard, 1987). This temperature manipulation is easy to achieve in captive/artificial incubation settings, so the PVA models were run such that all introduced juveniles were females, to increase the value of the supplemented turtles to the population as a whole. With this recovery strategy implemented, the MBPP Blanding's turtle population exhibited a 0% probability of extinction in the projections of 25 and 50 years but resumed a negative growth rate when population supplementation ceased, likely due to few introduced turtles reaching adult body size as a result of high juvenile mortality (Congdon et al., 1993), which is consistent with the work of Kuhns (2010). Based on these findings,

supplementation of juvenile Blanding's turtles is a more effective recovery strategy than nest protection efforts, but head-starting has its own limitations. The resources and effort that go into head-starting programs may be in vain because it is unclear whether the released turtles are able to successfully forage, mate, and nest. Dodds et al. (1991) explain that head-starting programs typically attract media attention and are favored by the public, but that there is a lack of evidence of failed head-starting initiatives, and there is also limited commitment to long-term monitoring of reintroduced populations or individuals, which is key in determining the success of these programs for long-lived species.

However, there is some evidence of successful reintroductions of turtles in North America. The introduction of Blanding's turtle juveniles into the Rouge National Urban Park was first done by the Toronto Zoo in June 2014 (10 individuals), and again in June 2015 (21 individuals), and will continue in successive years. Of the 10 juveniles that were released in 2014, one remains alive, while 19 of the 21 individuals released in 2015 survived their first winter in the wild (Yannuzzi, pers. comm., 2016; Toronto Zoo, 2015). Additionally, Roth and Krochmal (2015) found that naïve painted turtles (*C. picta*) that are younger than 4 years of age were able to navigate a landscape by following paths used by adults to find adequate overwintering sites and other important areas, such as food and water sources. The findings presented by the Toronto Zoo (2015) and Roth and Krochmal (2015) offer hope for the potential of juvenile supplementation into failing Blanding's turtle populations. However, the risk of ultimate population failure following introductions of juveniles is reinforced by Heppell et al. (1996), who state that even when head-starting programs are deemed successful, small decreases in adult survivorship quickly overcome the benefits of the head-starting program. Despite these risks, supplementation

of juveniles into MBPP main fen Blanding's turtle deme has potential to aid the persistence of the population into the future.

### *Introduction of Adults*

Models indicate that supplementation of adults into the MBPP Blanding's turtle population results in a long lasting positive effect on population persistence. The probability of extinction remained 0% in projections up to 75 years into the future but rose to and then exceeded 50% in projections between 100 and 125 years into the future. Similar to the "introduction of juveniles" scenario, the model shows population increases for the duration of the supplementation period, resuming a negative population growth rate when supplementation ceased. This pattern has been encountered in similar modeling cases (Kuhns, 2010). Due to the long amount of time required for Blanding's turtles to reach sexual maturity (at least at 14 years and up to 25 years of age in the northern regions of their range (COSEWIC 2005; Bury and Germano, 2003), it would be extremely costly, space-consuming, and difficult to rear this species into adulthood for the purpose of release into wild populations. Because adult survivorship plays a large role in population success, scenarios in which adults were supplemented into the MBPP Blanding's turtle population were explored, as has been done in other studies (Enneson and Litzgus, 2008), but in reality this is not a practical strategy.

Furthermore, any adults introduced to the MBPP habitat, whether they were raised in captivity or translocated from another area, may be unable to find adequate overwintering or nesting locations, which are essential for their individual survival, and ultimately the persistence of the population. In another study, the ability of naïve painted turtles to follow the paths of adults

when navigating a new landscape was lost by age 4, which is much earlier than sexual maturity (Roth and Krochmal, 2015). Finally, the absence of studies in the primary literature involving the introduction of adult turtles into sites with declining populations reaffirms that this strategy lacks real-world applications. Ultimately, the introduction of adults into MBPP is likely too costly and time consuming to be sustainable, and also has a high risk of failure, and so this strategy is not recommended for the recovery of the MBPP Blanding's turtle population.

#### *Combination of Nest Protection and Introduction of Juveniles*

Combining two of the previously discussed strategies, nest protection and the introduction of juveniles, proved to be the most effective recovery strategy to ensure persistence of Blanding's turtles in MBPP. In projections up to 250 years into the future, the probability of extinction did not surpass 65%. This strategy is also favoured because it led to population success with 50% fewer juveniles introduced at each supplementation event as compared to the "Introduction of Juveniles" strategy, both of which occurred annually for 25 years. It also allows for the release of turtles who are naïve and likely able to learn about their environment, which is less likely when considering the introduction of adults into MBPP. It seems that low reproductive frequency paired with high hatchling mortality are the major challenges for the MBPP Blanding's turtles, which are both addressed through this combination recovery strategy.

#### *Cautions Regarding Interpretations of Population Viability Analyses*

While PVAs are powerful and useful tools for estimating the success or failure of populations of various species, each PVA is unique and the results must be interpreted with some caution (Akçakaya and Sjogren-Glove, 2000). While much of the data incorporated into the PVAs of my

study relate specifically to the Blanding's turtles in MBPP, gained over three field seasons, there was also a considerable amount of data incorporated from other sources, which may or may not adequately describe the dynamics of the MBPP Blanding's turtle population (Akçakaya and Sjogren-Glove, 2000). Conversely, the use of information from other populations increases the potential for my results to be applied elsewhere, not limiting them solely to the MBPP Blanding's turtle population.

To achieve more population-specific information, long term studies focusing on MBPP are needed (Congdon et al., 1993). I have erred on the conservative side when determining input values for my PVAs, including female reproductive frequency, to reduce the chance of obtaining deceptively optimistic results. While absolute values are helpful in describing the effects of various parameters on the population, it is more important to compare trends between and within scenarios. Each PVA represents what is known at a particular point in time, and should be routinely updated as additional and more modern information is acquired, and as populations experience unanticipated changes in the future, whether they be of natural or anthropogenic origin (Middleton and Chu, 2004).

#### *Recommendation to Aid the Recovery of Blanding's Turtles at Misery Bay Provincial Park*

Populations of long-lived vertebrates, such as Blanding's turtles, lack density-dependent responses to increased mortality rates, and as such, are especially sensitive to an increased loss of adults from a population (Congdon et al., 1994; Brooks et al., 1991), as occurred through the MBPP MME in 2012. This information, paired with the aforementioned PVA findings, highlight the necessity of recovery strategies to be implemented on this population of at-risk Blanding's turtles, who will undoubtedly experience local extinction if left unaided.

Based on the findings presented, it is clear that some of the evaluated recovery strategies offer less risk than others in terms of achieving population success and persistence into the future. Nest protection offers relatively little assurance of success, and the challenges associated with adult rearing in captivity, and the potential lack in the ability of adults to survive when introduced to a new habitat, indicate that these initiatives would be quite risky and may not offer any relief to the depleted MBPP Blanding's turtle population. While there are also risks associated with juvenile rearing and introduction to new habitats (Kuhns, 2010; Dodd et al., 1991), there is evidence to suggest that this initiative would be worthwhile (Mitrus, 2005). Success of juvenile supplementations is reported in the literature, and the rearing of young Blanding's turtles has been successful at institutions such as the Toronto Zoo (released in Rouge National Urban Park, Toronto, Ontario), Oakland Farm Zoo (released in Nova Scotia), and Stone Zoo (released in Concord, Massachusetts). Young turtles are likely able to successfully navigate new landscapes, and the PVAs conducted here indicate that successful reintroductions have the potential to aid in population persistence well into the future. The most optimistic scenario detailed a combination of nest protection and the introduction of female juveniles, with far fewer juveniles than were included in the strategy that involved only introduction of juveniles. This leads me to recommend that, should recovery action be taken, the protection of nests paired with the supplementation of female juveniles should be implemented as a recovery strategy for the Blanding's turtles in MBPP, whose population suffered unprecedented adult mortality in 2012.

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## Tables, Figures, and Appendices

### Tables

**Table 2.1:** Quasi Akaike's Information Criterion (QAICc) values for comparing POPAN population models in Program MARK, used to estimate the population size of the remaining Blanding's turtles (*Emydoidea blandingii*) in the main fen area of Misery Bay Provincial Park, Ontario. The Constant Survival model ranked highest, based on QAICc values.

Model	QAICc Value
Constant Survival	80.4
Fully Time Dependent	82.9
Constant Survival and Probability of Entry	91.1

**Table 2.2:** Parameters used for the base model in the population viability analyses, including life history characteristics of Blanding’s turtles (*Emydoidea blandingii*). The base model represents the current population, which experienced a mass mortality event (MME) with no further conservation strategy applied.

Parameter	Values	Source
Initial Population Size	100 (main fen) 13 (SW corner)	This study
Breeding Adult Females (%)	25 (main fen) 37.5 (SW corner)	This study
Breeding System	Polygynous	Refsnider, 2009
Breeding Adult Males (%)	100	Ernst et al., 2009
Maximum Number of Clutches Per Year	1	This study
Maximum Clutch Size (eggs)	15	Standing et al., 2000
Sex Ratio at Hatch	1:1	-
Male Age at First Reproduction	20	COSEWIC, 2005
Female Age at First Reproduction	20	COSEWIC, 2005
Maximum Age of Reproduction (both sexes)	83	Science Daily, 2016
Maximum Lifespan	83	Science Daily, 2016
Mortality (%; age 0-1)	74	Congdon et al., 1993
Mortality (%; age 1-13)	22	Congdon et al., 1993
Mortality (%; age 14+)	4	Congdon et al., 1993
Harvest	1M and 1F every 3 years (main fen) 1M and 1F every 5 years (SW corner)	-
Quasi Extinction Threshold (number of individuals)	8	Enneson and Litzgus, 2009
Dispersal	4 individuals from SW corner to main fen	This study
Density Dependence	No	Brooks et al., 1991
Density Dependent Reproduction	No	Brooks et al., 1991
Carrying Capacity	500 individuals	-

**Table 2.3:** Summary of input values for the population viability analyses conducted based on several scenarios, including the base model (in which a mass mortality event (MME) occurred and the population was unaided), no-MME, nest protection, introduction of juveniles, and introduction of adults as conservation strategies hypothetically implemented on the main fen Blanding’s turtle population of Misery Bay Provincial Park, Ontario.

Parameter	Scenario Names and Associated Values						Source
	Base Model (No Conservation Initiatives Implemented)	No MME	Nest protection (Main Fen)	Introduction of Juveniles (Main Fen)	Introduction of Adults (Main Fen)	Nest protection + Intro. of Juv. (Main Fen)	
Initial Population Size	47	100	47	47	47	47	This study
Mortality (%; age 0-1)	74	74	0	74	74	0	Congdon et al., 1993
Number of Juveniles Supplemented	0	0	0	50	0	25	Toronto Zoo, 2015
Number of Adults Supplemented	0	0	0	0	25	0	-

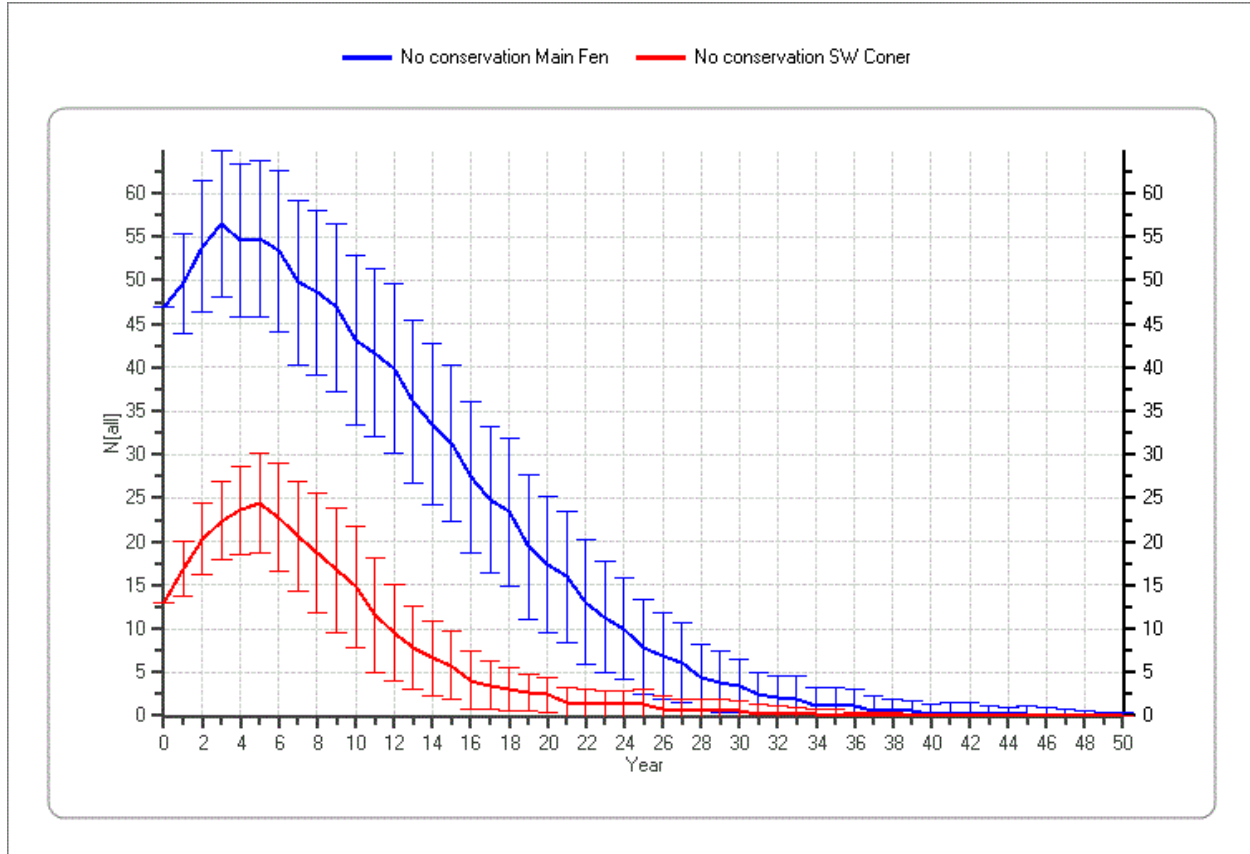
**Table 2.4:** Summary of output values from the population viability analyses conducted based on several scenarios, including the base model (in which a mass mortality event (MME) occurred and the population was unaided), no-MME, and nest protection, introduction of juveniles, and introduction of adults as conservation strategies hypothetically implemented on the Blanding’s turtle population in the main fen area of Misery Bay Provincial Park, Ontario. Each scenario was run with 500 iterations, and detailed in this table are projections of various timespans into the future. “Years” represent the number of years into the future that the summary statistics represent. The quasi-extinction threshold was set at 8 individuals for each model. “N-extant” is the number of individuals remaining in the population at the end of each timespan in iterations that did not result in extinction.

Scenario	Probability of Extinction (%)	N-extant	SD(N- extant)	r	SD(r)
Base Model (25 years)	57	13	4.55	-0.066	0.139
Base Model (50 years)	100	8	0.00	-0.068	0.142
No MME (25 years)	0	54	14.45	-0.026	0.097
No MME (50 years)	90	13	4.47	-0.055	0.118
No MME (75 years)	100	0	0.04	-0.056	0.118
Nest protection (25 years)	1	46	24.02	-0.010	0.196
Nest protection (50 years)	71	25	15.69	-0.035	0.203
Nest protection (75 years)	99	43	35.04	-0.038	0.204
Nest protection (100 years)	100	0	0.04	-0.040	0.205
Introduction of Juveniles (25 years)	0	250	15.05	0.067	0.149
Introduction of Juveniles (50 years)	0	38	13.71	-0.005	0.144
Introduction of Juveniles (75 years)	53	12	5.54	-0.022	0.132
Introduction of Juveniles (100 years)	100	0	0.16	-0.024	0.132
Introduction of Adults (25 years)	0	32	7.75	-0.020	0.216
Introduction of Adults (50 years)	0	62	16.19	0.004	0.222
Introduction of Adults (75 years)	0	54	16.94	0.001	0.200

Introduction of Adults (100 years)	18	12	5.70	-0.015	0.180
Introduction of Adults (125 years)	94	20	10.75	-0.016	0.174
Introduction of Adults (150 years)	97	15	6.98	-0.017	0.173
Introduction of Adults (175 years)	99	10	2.31	-0.017	0.174
Nest protection + Intro of Juv. (25 years)	0	166	23.46	0.050	0.155
Nest protection + Intro of Juv. (50 years)	0	115	81.52	0.011	0.163
Nest protection + Intro of Juv. (75 years)	20	108	75.06	0.001	0.175
Nest protection + Intro of Juv. (100 years)	50	92	74.42	-0.009	0.176
Nest protection + Intro of Juv. (125 years)	53	86	65.32	-0.009	0.176
Nest protection + Intro of Juv. (150 years)	59	93	73.42	-0.009	0.178
Nest protection + Intro of Juv. (175 years)	53	98	74.46	-0.007	0.172
Nest protection + Intro of Juv. (200 years)	59	99	66.26	-0.007	0.174
Nest protection + Intro of Juv. (250 years)	64	94	71.89	-0.008	0.177



## Figures



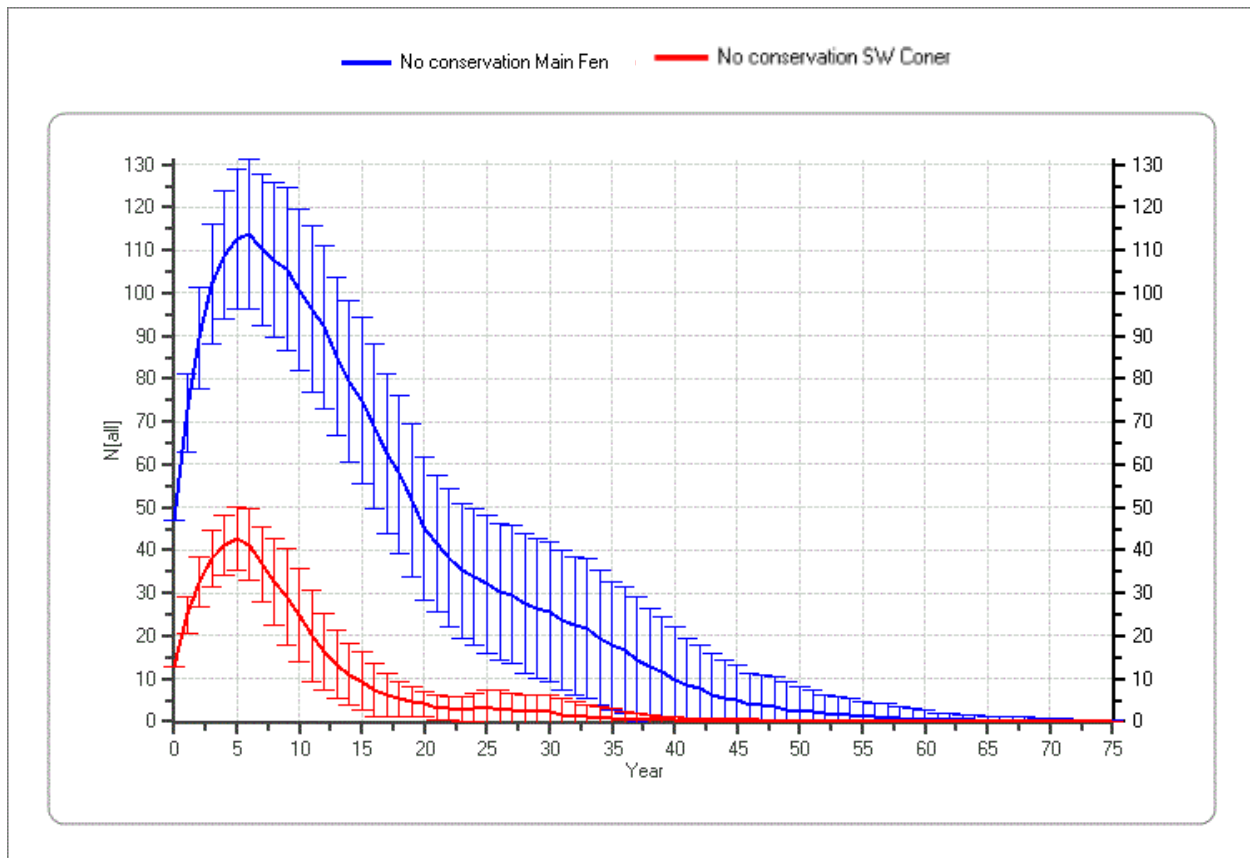
**Figure 2:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 50 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. This base model describes the life history parameters of Blanding's turtles, including those of the Main Fen study population as experiencing a mass mortality event (MME) without the aid of any post-MME recovery actions. Error bars represent the standard deviation of the mean number of individuals in the population each year.

## Appendices

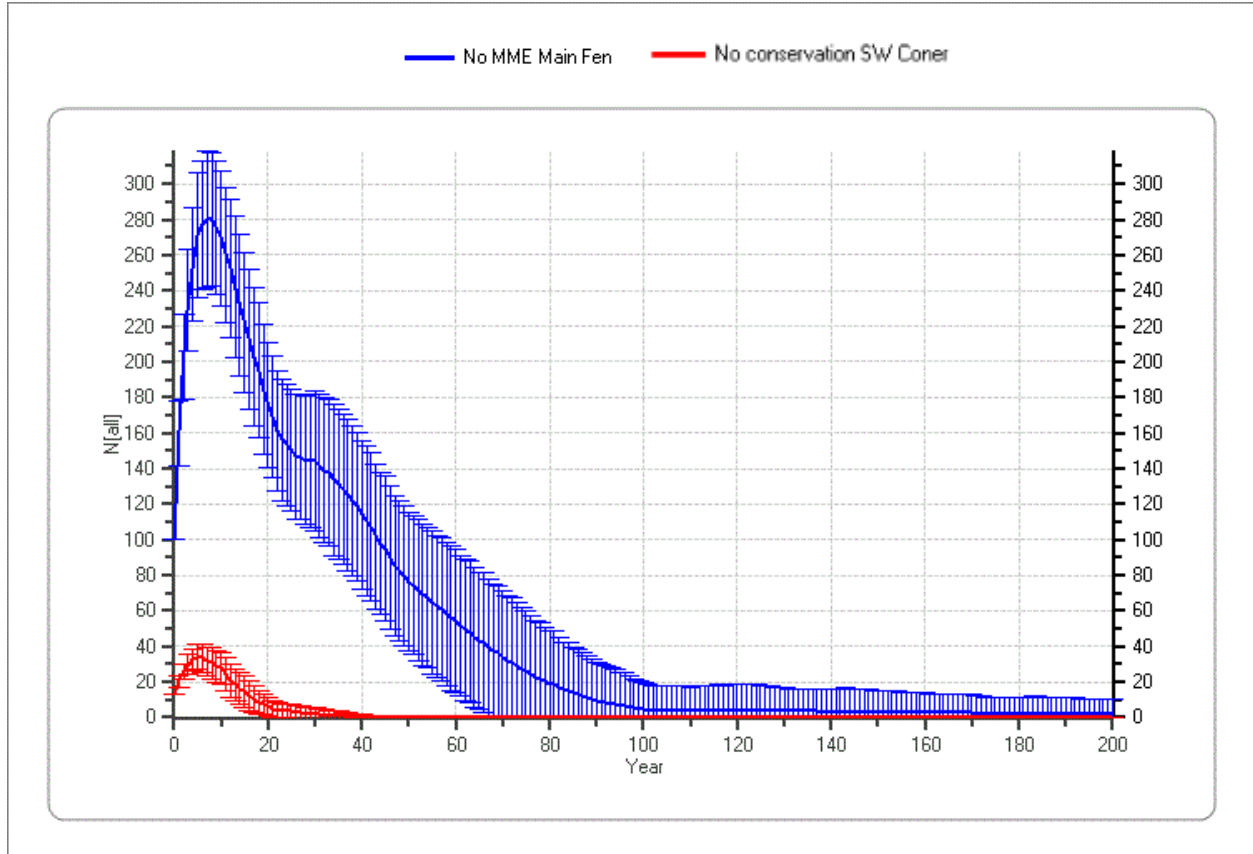
### Appendix II

**Appendix II.I:** Parameters used for the base model in the population viability analyses, including life history characteristics of Blanding's turtles (*Emydoidea blandingii*). The base model represents the current population with a high proportion of breeding females, which experienced a mass mortality event (MME) with no further conservation strategy applied.

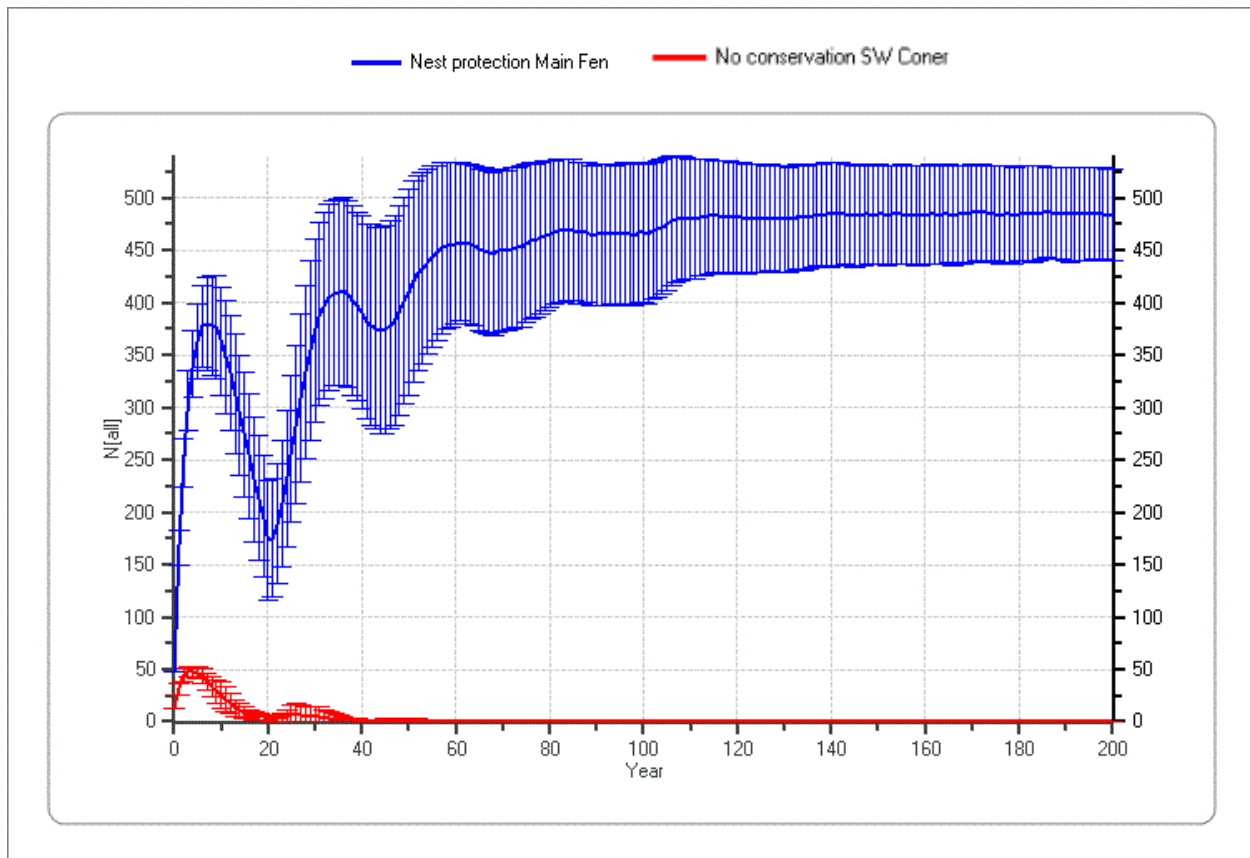
Parameter	Values	Source
Initial Population Size	100 (main fen) 13 (SW corner)	This study
<b>Breeding Adult Females (%)</b>	<b>80 (main fen)</b> <b>80 (SW corner)</b>	Congdon et al., 1993
Breeding System	Polygynous	Refsnider, 2009
Breeding Adult Males (%)	100	Ernst et al., 2009
Maximum Number of Clutches Per Year	1	This study
Maximum Clutch Size (eggs)	15	Standing et al., 2000
Sex Ratio at Hatch	1:1	-
Male Age at First Reproduction	20	COSEWIC, 2005
Female Age at First Reproduction	20	COSEWIC, 2005
Maximum Age of Reproduction	83	Science Daily, 2016
Maximum Lifespan	83	Science Daily, 2016
Mortality (%; age 0-1)	74	Congdon et al., 1993
Mortality (%; age 1-13)	22	Congdon et al., 1993
Mortality (%; age 14+)	4	Congdon et al., 1993
Harvest	1M and 1F every 3 years (main fen) 1M and 1F every 5 years (SW corner)	
Quasi Extinction Threshold (number of individuals)	8	Enneson and Litzgus, 2009
Dispersal	4 individuals from SW corner to main fen	This study
Density Dependence	No	Brooks et al., 1991
Density Dependent Reproduction	No	Brooks et al., 1991
Carrying Capacity	500 individuals	-



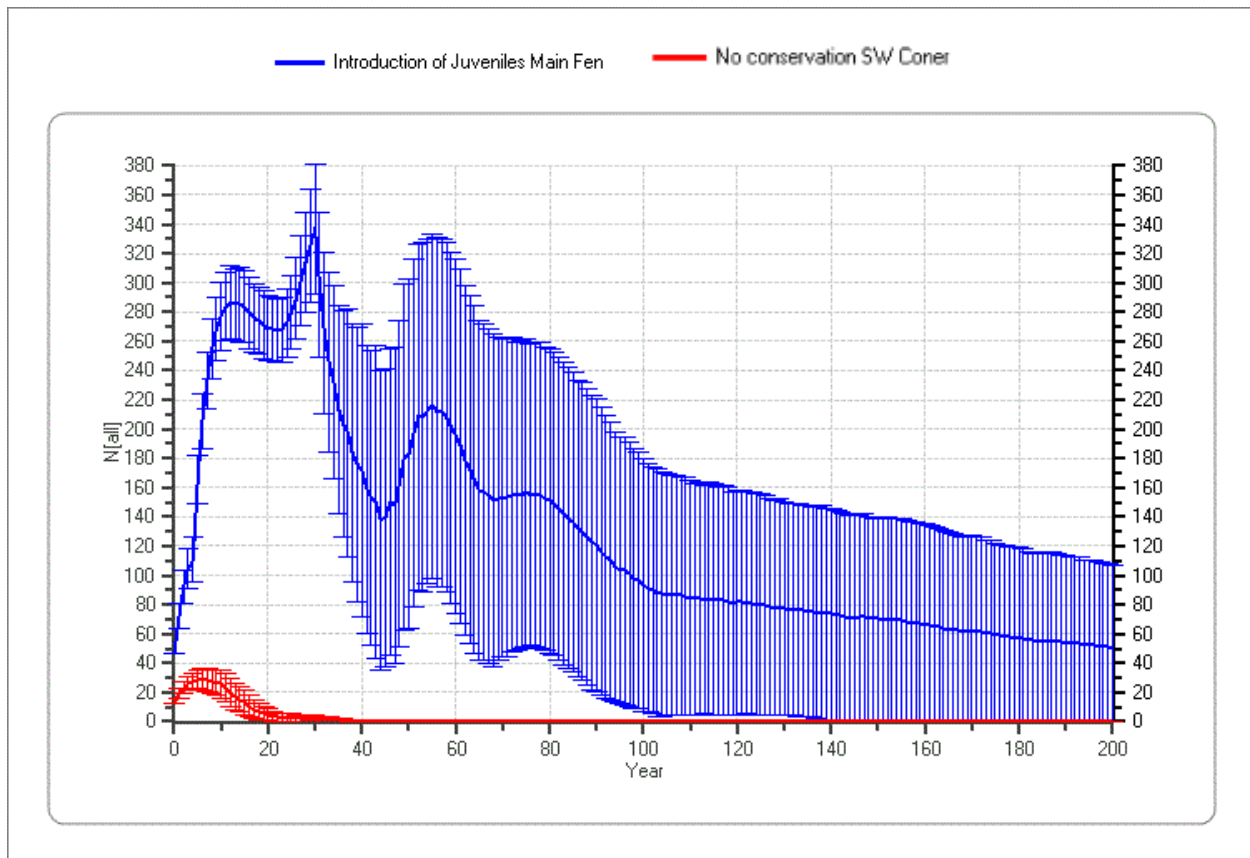
**Appendix II.IIa:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 50 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. This base model describes the life history parameters of Blanding's turtles with an increased proportion of breeding females (80%), including those of the Main Fen study population as experiencing a mass mortality event (MME) without the aid of any post-MME recovery actions. Error bars represent the standard deviation of the mean number of individuals in the population each year.



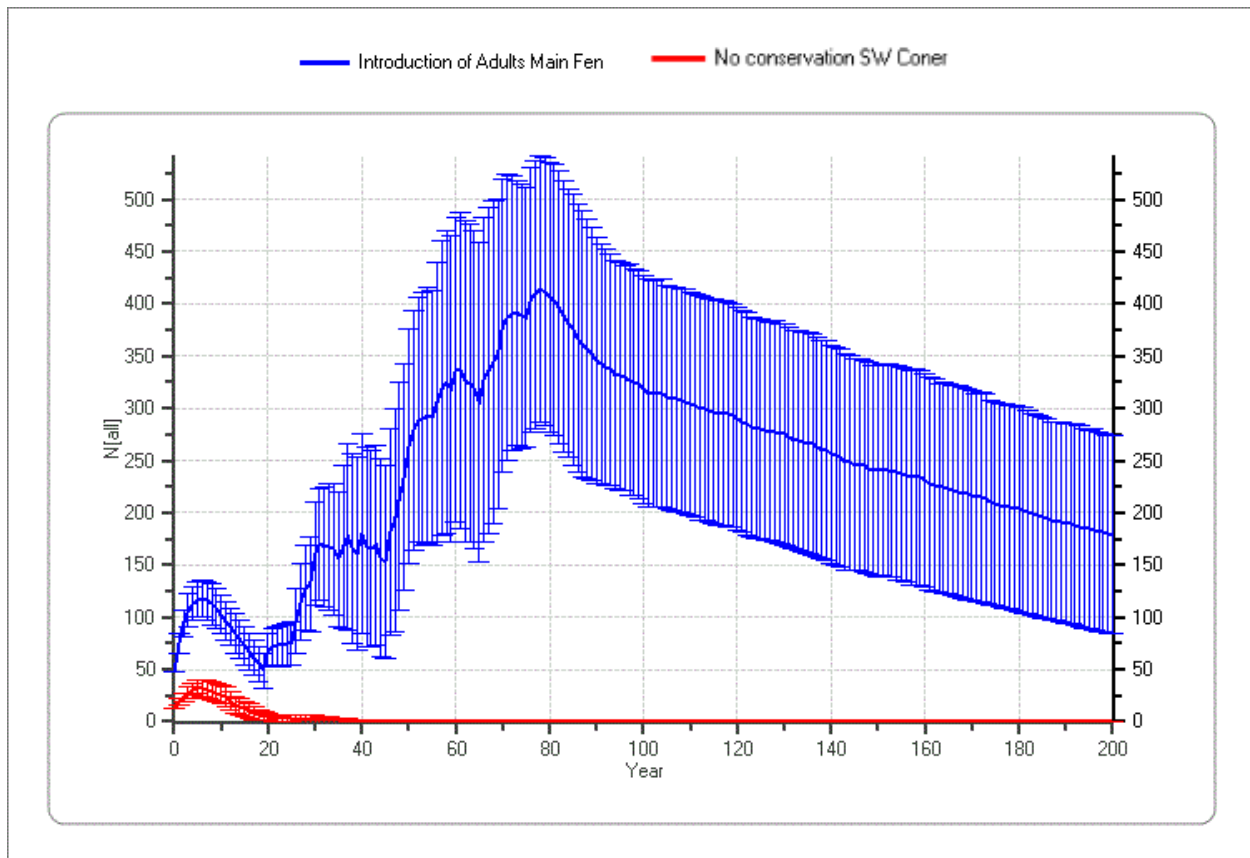
**Appendix II.IIb:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 75 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. This model describes the life history parameters of Blanding's turtles with an increased proportion of breeding females (80%), including those of the study population as though it never experienced a mass mortality event (MME). Error bars represent the standard deviation of the mean number of individuals in the population each year.



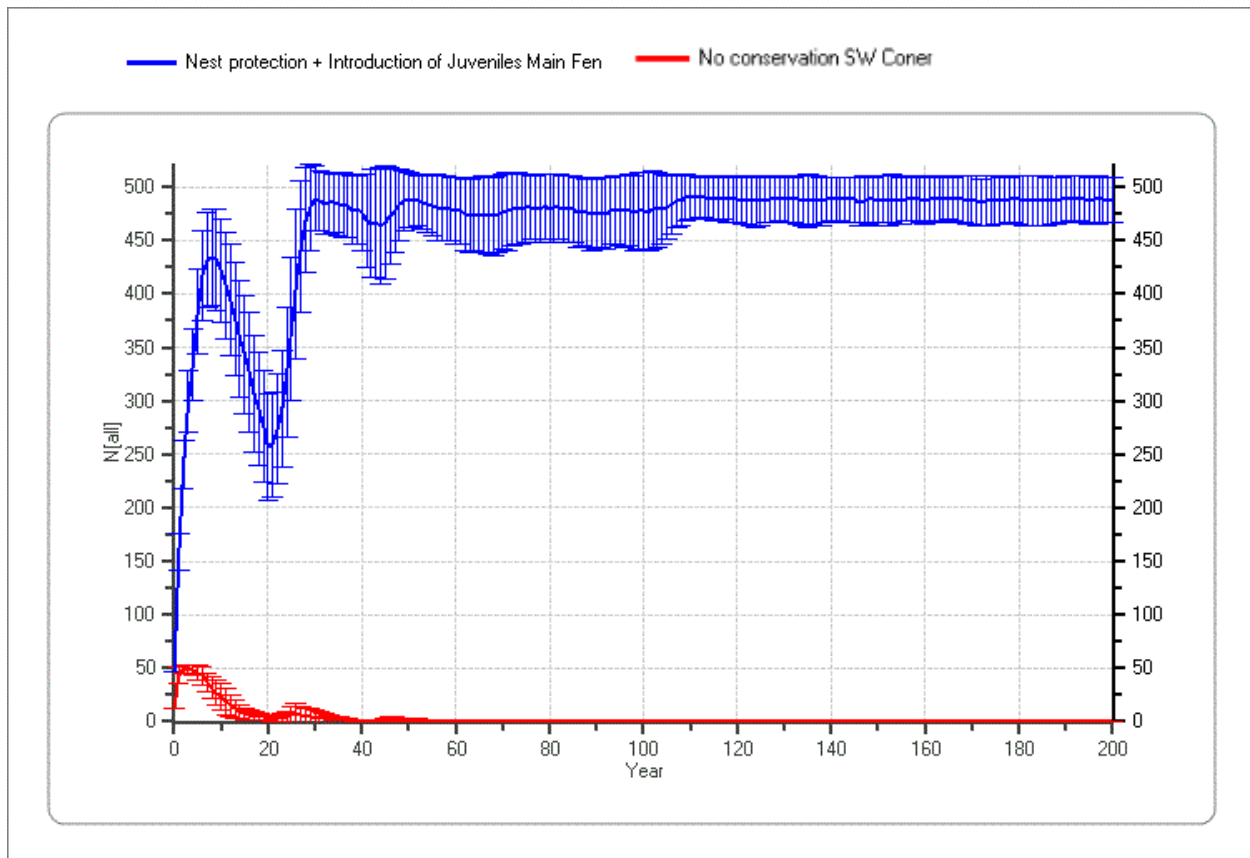
**Appendix II.IIc:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 100 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. This model describes the life history parameters of Blanding's turtles with an increased proportion of breeding females (80%), including those of the Main Fen study population as though it experienced a mass mortality event (MME), and also nest protection (survival at age 0-1 = 1.00) as a recovery strategy. Error bars represent the standard deviation from the mean number of individuals in the population each year.



**Appendix II.IId:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 100 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. his model describes the life history parameters of Blanding's turtles with an increased proportion of breeding females (80%), including those of the study population as though it experienced a mass mortality event (MME), and the annual introduction of 50 two-year-old juvenile females to the Main Fen area for a period of 25 years (beginning in year 5 and ending in year 30) as a recovery strategy. Error bars represent the standard deviation of the mean number of individuals in the population each year.



**Appendix II.IIe:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 175 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. his model describes the life history parameters of Blanding's turtles with an increased proportion of breeding females (80%), including those of the study population as though it experienced a mass mortality event (MME), and the annual introduction of 25 adults (15 years of age) into the Main Fen area every 10 years, beginning in year 20 and ending in year 70 as a recovery strategy. Error bars represent the standard deviation from the mean number of individuals in the population each year.



**Appendix II.IIf:** Projected mean exponential population growth (500 iterations) of Blanding's turtles (*Emydoidea blandingii*), as projected 250 years into the future at two sites (Main Fen and Southwest (SW) Corner) within Misery Bay Provincial Park, Ontario. This model describes the life history parameters of Blanding's turtles with an increased proportion of breeding females (80%), including those of the study population as though it experienced a mass mortality event (MME), and also nest protection of the Main Fen nests (survival at age 0-1 = 1.00) and the annual introduction of 25 two-year-old juvenile females to the Main Fen area for a period of 25 years (beginning in year 5 and ending in year 30) as a paired recovery strategy. Error bars represent the standard deviation of the mean number of individuals in the population each year.



## Appendix II.III

### **Blanding's Turtle Survey Protocol Misery Bay Provincial Park Manitoulin Island, Ontario**

#### **Primary Contacts**

**Name:** Ed Morris  
**Affiliation:** Ontario Parks, Northeast Zone  
**Position:** Ecologist  
**Phone:** 705-564-3162  
**Email:** edward.morris@ontario.ca

**Name:** Gaynor Orford  
**Affiliation:** Friends of Misery Bay  
**Position:** Chair, Head Volunteer  
**Phone:** 705-282-3035  
**Email:** orfordfa@vianet.ca

**Name:** Anna Sheppard  
**Affiliation:** Ontario Parks  
**Position:** Assistant Ecologist  
**Phone:** 705-564-7014  
**Email:** anna.sheppard@ontario.ca

**Name:** Dr. Jacqueline Litzgus  
**Affiliation:** Laurentian University  
**Position:** Biology Professor  
**Phone:** 705-675-1151, ext 2314  
**Email:** jlitzgus@laurentian.ca

**Name:** Donnell Gasbarrini  
**Affiliation:** Laurentian University  
**Position:** Master of Science Student 2014-16  
**Phone:** 905-977-8892  
**Email:** donnell.gasbarrini@gmail.com

#### **Purpose of Routine Surveys**

Routine surveys, primarily for Blanding's turtles, secondarily for painted and snapping turtles, have been recommended to occur in Misery Bay Provincial Park as a means of population monitoring following an unprecedented mass mortality event. The information from these surveys will be informative of any unusual events (such as another mortality event), habitat quality fluctuations, and can be used to determine population size estimates and to inform additional population viability modeling in the future.

### **General Overview**

Blanding's turtles found during these surveys are to be marked through notching of the posterior marginal scutes using the key on the sample datasheet below, so that they may be recognized as individuals if found during subsequent search events. Datasheets (sample provided below) will be filled in in full upon the first capture of each turtle of that year. Information regarding subsequent captures in the same year are to be filled out in the abbreviated version of the datasheet (sample provided below). Each turtle is to be processed in the field and released at the site of capture.

### **Recommended Time of Year**

Regular annual surveys should occur during the spring season, particularly during the month of May. Blanding's turtles are known to have large home ranges, and will disperse far from their overwintering sites during the summer months. In addition, the turtles are easier to see before the vegetation is up. As such, the best time of year to capture resident Blanding's turtles is during the spring. Blanding's turtles at Misery Bay Provincial Park have been captured in largest numbers at this time of year, as they are basking in close proximity to their overwintering sites, ridding their bodies of waste accumulated during the winter season.

### **Alternative Time of Year**

Instead of spring surveys, fall surveys (month of September/October) would also have somewhat high chances of locating Blanding's turtles in Misery Bay Provincial Park, as resident turtles congregate in areas surrounding overwintering sites.

## **Nesting Season**

To date, nest sites of resident turtles have all be located outside of Misery Bay Provincial Park, therefore landowner permission should be gained before attempting to survey for nesting Blanding's turtles.

## **Participants**

Due to the sensitive nature of the fen habitat in Misery Bay Provincial Park, and concerns about poaching of at-risk turtle species, participants must be approved by the aforementioned primary contacts. Events will not be open to the public, and as such will not be advertised in newspapers, on the radio, or on any social media platforms without the consent of the primary contacts.

## **Processing Procedure**

### **Notching**

Notches are marks made on the edges of the marginal scutes at the posterior end of the carapace. They follow the pattern as described below on the “first annual encounter” datasheet. They are to be made with triangular files, and the number of notches on each turtle should not exceed three until all possible combinations have been used, to reduce stress to the turtle and to aid in the ease of reading each turtle's number.

The ID number of each turtle is to be written numerically (ie. T004F is “turtle 004, a female”), and also using the notch placement, in which case T004F would be recorded as R11, as her only notch would be present on the 11<sup>th</sup> marginal scute, on the right side of her carapace.

### Turtle Mass

Each turtle should be weighed using a bag (preferably mesh or cloth) and a Pesola scale. The weight of the turtle is calculated by subtracting the weight of the bag from that of the combined weight of the turtle and the bag. Mass is to be recorded in grams.

### Measuring

**Maximum Carapace Length:** the longest straight-line distance from the anterior to posterior end of the carapace, measured in cm to two decimal places, if possible.

**Maximum Plastron Length:** the longest straight-line distance from the anterior to posterior end of the plastron, measured in cm to two decimal places, if possible.

**Midline Carapace Length:** the straight-line distance down the center of the carapace, beginning at the nuchal and ending between the 12<sup>th</sup> marginal scutes on each side, measured in cm to two decimal places, if possible. It is possible that the maximum and midline carapace lengths will be the same, depending on the shape of the shell.

**Midline Plastron Length:** the straight-line distance down the center of the plastron, following the center suture between the left and right plastral scutes, measured in cm to two decimal places, if possible. It is possible that the maximum and midline plastron lengths will be the same, depending on the shape of the shell.

**Carapace Height:** measured in line with the bridge suture on the ventral surface of the plastron, using calipers, to determine the height of the shell at this point along the body.

### Age

The age of a turtle can be estimated by counting growth rings, which are most obvious on the plastron of young turtles. Older turtles may have reduced, or no, evidence of these growth rings. If present, the number of rings should be recorded, and a photograph taken for future reference. Whether or not the growth rings are worn smooth should also be recorded.

### Sex

Female Blanding's turtles have a flat plastron, while that of males is concave. Females also have much thinner/smaller tails than males, whose tails are thicker and also have the cloaca positioned farther away from the end of the plastron, all for mating purposes. The easiest way to tell is to look at the plastron, as tail characteristics can be difficult without direct comparison between the sexes. The sex of juvenile turtles cannot be determined.

### Temperature

Air temperature is to be recorded in degrees Celsius, using a thermometer held in the shade at approximately 1.5m above the surface of the ground or water. Readings will not be accurate if the thermometer is held in direct sunlight while readings are being taken. Water temperature is to be taken approximately 20-25cm below the surface of the water.

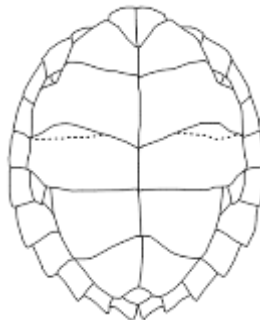
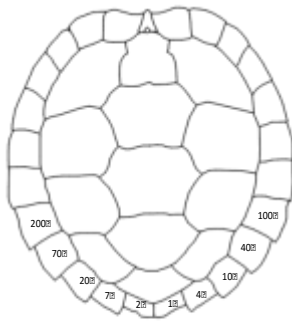
### Sample Datasheet- First Annual Encounter

<b>Date</b>	<b>Time</b>	<b>Notch #</b> <div style="text-align: right; font-size: small;">existing new</div>	<b>Species</b>
<b>Area of Park</b>		<b>Locality</b>	
<b>Weather</b>	<b>Precip</b>	<b>Beaufort</b> 0 1 2 3 4 5 6+	<b>%Cloud</b>
<b>UTM (Nad83)</b> <div style="text-align: right; font-size: small;">Accuracy</div>		<b>Waypoint</b>	<b>Transmitter Freq.</b>
			<b>Water Temp (°c)</b>
			<b>Distance to water/land</b> (m)

<b>Max Carapace Length (mm)</b>	<b>Max Carapace Width (mm)</b>
<b>Mid Carapace Length (mm)</b>	<b>Carapace Height (mm)</b>
<b>Max Plastron Length (mm)</b>	<b>Mass (g)</b>
<b>Mid Plastron Length (mm)</b>	<b>Age estimate (yrs)</b>

<b>Sex</b> M    F    J    Unk	<b>Gravid</b> Yes   No   Unk   N/A	<b>Blood Sample</b> Yes   No	<b>Photo</b> Yes   No <b>ID:</b>
<b>Carapace Annuli</b>	<b>Wear</b> none   light   mod   ext	<b>Plastron annuli</b>	<b>Wear</b> none   light   mod   ext

<b>Substrate</b> Sand   gravel   muck   grasses/sedges   leaf litter   mud   moss   twigs   other:			
<b>Habitat</b>	<b>Water Depth</b>	<b>Nearest aquatic habitat</b>	<b>Nearest forest/vegetation type</b>
<b>Activity/behaviour</b> basking   resting   in water   walking   mating feeding   nesting   other:			<b>Direction of travel</b>
<b>Injuries</b> none   tail   eyes   legs   carapace   plastron   other:		<b>Injury description:</b>	
<b>Deformities:</b>		<b>Leeches:</b>	
<b>How Found:</b> Telemetry   General Search   Other:			
<b>Additional Comments &amp; Sampler Names:</b>			

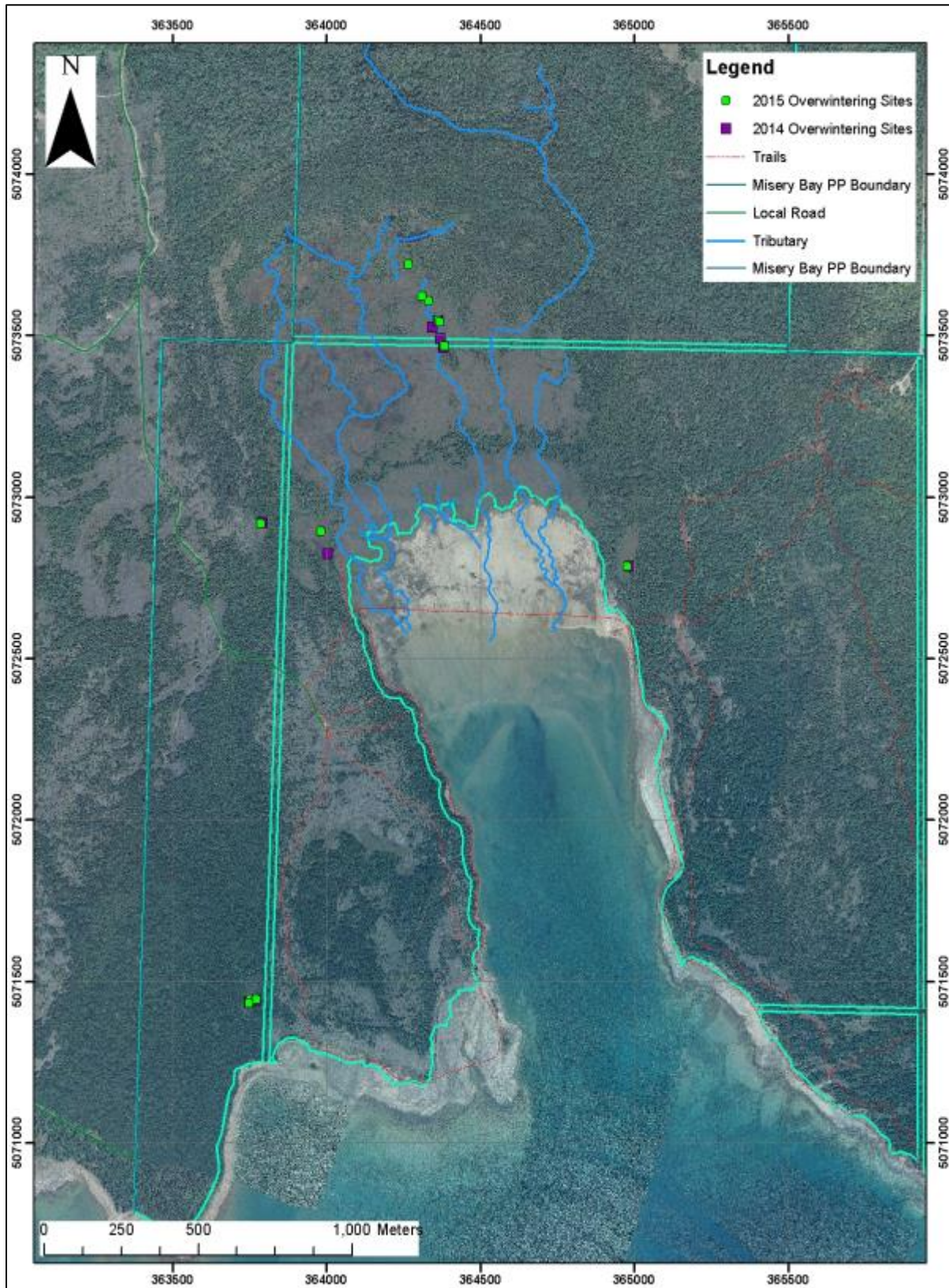


**Beaufort Wind Scale:** 0. Calm, smoke rises vertically 1. Light air movement, smoke drifts 2. Slight breeze, wind felt on face 3. Gentle breeze, small twigs move 4. Moderate breeze, small branches move 5. Fresh breeze, small trees sway.

Sample Datasheet: Subsequent Encounters (can repeat table up to 3x per page)

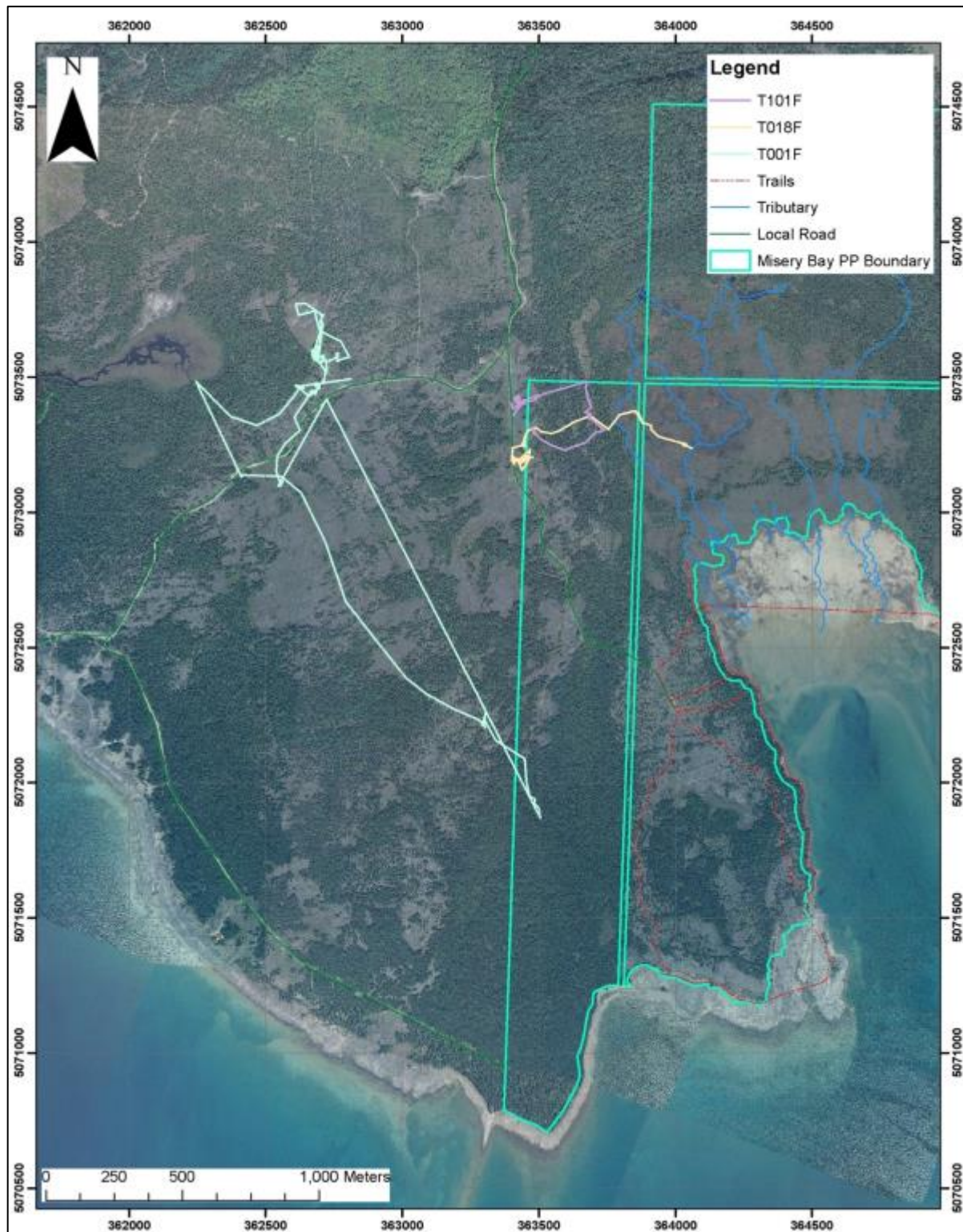
<b>Date</b>	<b>Time</b>	<b>Notch #</b>	<b>Air Temp</b>	<b>Water Temp</b>	<b>Waypoint</b>
<b>Area of Park</b>	<b>Locality</b>	<b>Distance to water/land</b> (m)	<b>UTM (Nad83)</b>  Accuracy		
<b>Weather</b> Precip %Cloud	Beaufort	<b>Gravid</b> Yes No Unk N/A	<b>Blood Sample</b> Yes No	<b>Photo</b> Yes No <b>ID:</b>	
<b>Substrate</b> sand gravel muck grass/sedge leaf litter mud moss other:					
<b>Habitat</b>	<b>Water depth</b>	<b>Nearest aquatic type</b>	<b>Nearest forest/vegetation type</b>		
<b>Activity/behaviour</b> basking resting walking mating feeding nesting other:			<b>Direction of travel</b>		
<b>New Injuries</b> none tail eyes legs carapace plastron other		<b>Injury description</b>		<b>Leeches (count)</b>	
<b>How found</b> Telemetry General Search		<b>Remarks</b>			<b>Observer</b> <b>Initials</b>

**Beaufort Wind Scale:** 0. Calm, smoke rises vertically 1. Light air movement, smoke drifts 2. Slight breeze, wind felt on face. 3. Gentle breeze, small twigs move 4. Moderate breeze, small branches move 5. Fresh breeze, small trees sway.



The map shows the main fen and southwest corner area wetlands of Misery Bay proper, showing the overwintering locations of telemetry turtles in the winter of 2014 (purple) and 2015 (green).





The map shows the main fen and southwest corner area wetlands of Misery Bay proper, highlighting the routes of 3 nesting females, T001F (origin: southwest corner; light blue), T018F (origin: main fen; light orange), T101F (origin: east of Misery Bay Provincial Park; purple).

## **General Conclusion**

### *Misery Bay Mass Mortality Event*

With reptile declines occurring both locally and globally, affecting Ontario's native turtle species in particular, it is important to be aware of the threats to these species at risk, and the long-term effects that these threats will have on population, and ultimately specie persistence. The MME that occurred at Misery Bay Provincial Park in 2012 represents a unique case in which no direct anthropogenic link to the cause of increased mortality is apparent, as many of the well-known threats to turtles result directly from human expansion, development, and activity.

There remains much to learn about MMEs and the ways in which populations with long life history strategies are affected (Fey et al., 2015). This is especially clear when considering that the number of MMEs occurring in reptile populations has increased since the 1970s, but many remain unreported and/or unsolved (Fey et al., 2015). My study examined several potential causes of death, and determined that the cause is almost likely predation. While predators are known threats to turtles, the intensified role that they play in adverse environmental conditions has recently become clear in the literature, and possibly in the MBPP MME as well, which happened during a drought year (Fey et al., 2015; Cypher et al., 2014; Stacy et al., 2014; Anthonysamy et al., 2013; Gronewold et al., 2013; Rowe et al., 2013; COSEWIC, 2005; Aresco et al., 2003; Longshore et al., 2003; Reed et al., 2003; Hall and Cuthbert, 2000; Christiansen and Bickham, 1989; Ultsch, 1989; Gibbons et al, 1983). Furthermore, while these events are currently thought of as rare, it is likely that with increasing environmental stochasticity, they will occur more frequently. My study emphasizes the importance of routine wetland monitoring, especially in times of unusual environmental conditions. It also provides a comparison of

recovery strategies for the Blanding's turtles at MBPP, but can also be taken into consideration when managing long-lived species that have been affected by MMEs elsewhere. It is my hope that the scenarios described herein will be considered, and thus aid in the formation of a recovery strategy for the Blanding's turtles affected by a mass mortality event at Misery Bay Provincial Park.

### *The Role of Conservation Biology in the Wake of a Natural Die-Off*

Humans are, without doubt, responsible for many population declines that have been documented worldwide, and have assumed the responsibility of "saving species" through a field of science called conservation biology. Conservation biology emerged in the 1970s as field concerned with informing management and policy makers for the purpose of securing the persistence of floral and faunal species at risk of extinction (Van Houtan, 2005). With regards to the MME at MBPP, is there a role for conservation biologists to play, considering that my data strongly suggest that the cause of the MME was a natural predation event?

Without a first-degree human connection to the MME, in a protected habitat lacking the usual anthropogenic threats, and in a world where funding for conservation action is limited, whether or not it is the responsibility of humans to remedy the fate of the MBPP Blanding's turtles is unclear. The PVAs that I conducted indicate that the MBPP demes may very well have been in a state of decline before the MME. However, given what is known about the natural history of Blanding's turtles, what remains unknown about MMEs, and the challenges that Blanding's turtles face elsewhere in their Ontario range, I argue that biologists have a responsibility to the turtles at MBPP. While the PVAs showed ultimate declines in the various scenarios, including

that in which no MME took place, it is crucial to recall that population-specific information was not available for all parameters. Furthermore, some parameters, including female reproductive rates, were based on a small subset of the population, and may not be truly representative. As previously mentioned, the results must be viewed with caution, and the outcomes of the scenarios should be compared to one another rather than taken as absolutes. While limiting, this is the nature of the information that we have about this population of Blanding's turtles at this time.

Fieldwork conducted in MBPP revealed that resident turtles are not the only individuals who spend time in the park, and that some of the resident turtles leave the park for periods of time. As a provincially significant wetland of ~90 ha, the MBPP Blanding's turtles likely play an integral role in the metapopulation system, which would suffer greatly if the MBPP turtles experienced a local extinction. As such, the conservation of Blanding's turtles within the park will almost undoubtedly aid in preserving surrounding demes in this region of Manitoulin Island, Ontario. Likewise, existing on an island, these Blanding's turtles likely have unique genotypes as compared to those in the mainland or in disjunct populations, making them inherently valuable (Green, 2005).

If nothing else, it is important to continue to routinely monitor the turtles at MBPP, even if it is simply to determine the consequences of a loss of this magnitude in a protected habitat that is almost devoid of anthropogenic threats. Being situated a mere 221 km drive away from Sudbury, where herpetofaunal researchers Drs. Litzgus and Lesbarrères are located, and where both hubs for Ontario Parks and Ontario Ministry of Natural Resources and Forestry are located, there is no

lack of nearby qualified personnel to carry out such monitoring. A major challenge in determining the cause of death of these turtles is that the carcasses were not discovered until nearly an entire year after the mortality event took place, erasing many useful clues. Regular monitoring would increase the likelihood of noticing irregularities such as this MME before too much time had elapsed. Regular monitoring will also allow for baseline data to continue to be accumulated, regarding both the turtles and their habitat, which will aid in future studies. Finally, monitoring efforts will allow for a unique perspective on the role of a MME in the absence of additional pressures, which remains largely unknown for long lived organisms such as turtles. Recovery action would be beneficial for the Blanding's turtles in and surrounding MBPP, but there remains an obligation to continue to study these turtles and their habitat, even if direct conservation initiatives are not applied.

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